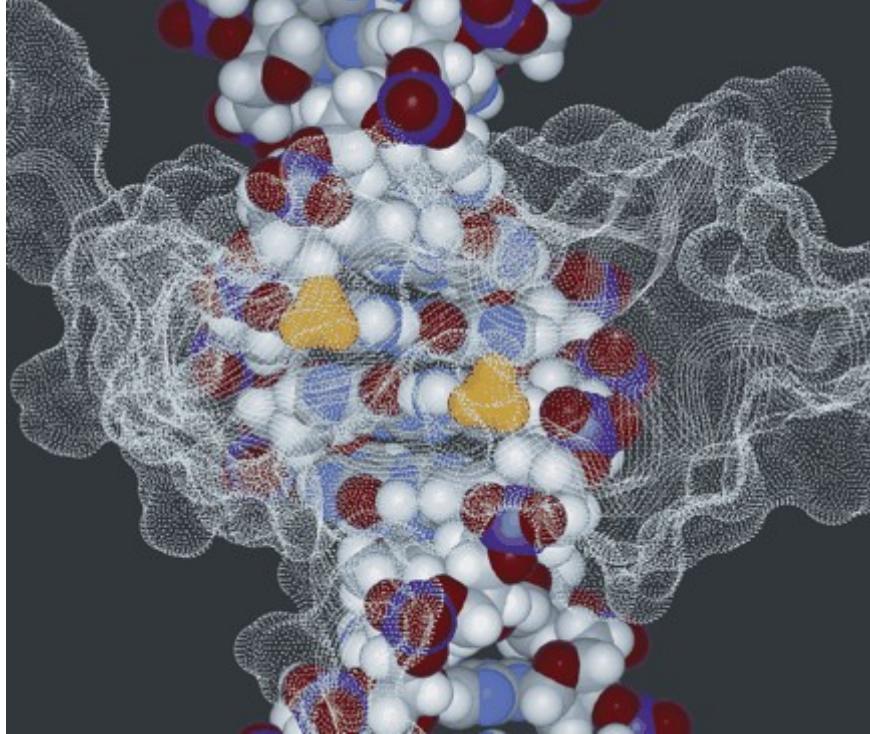


DNA methylation and gene activity in mammals (continued...)



Nucleic Acids Research (2004) 32, 4100-4108

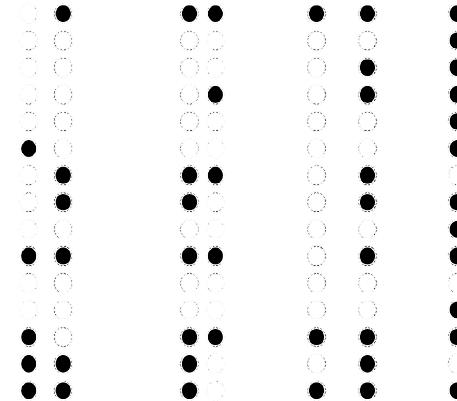
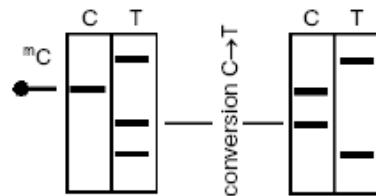
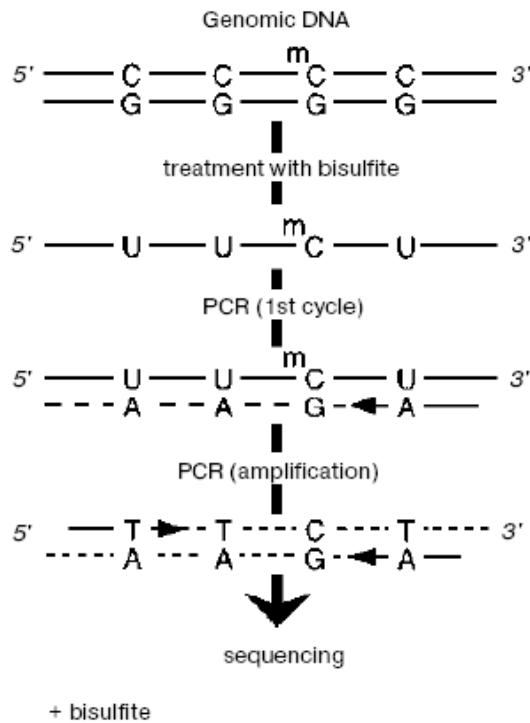
Miguel Constância

Senior Lecturer in Reproductive Biology

Metabolic Research Laboratories, Department of Obstetrics & Gynaecology
University of Cambridge; email: jmasmc2@cam.ac.uk

Mapping DNA methylation

- Bisulfite

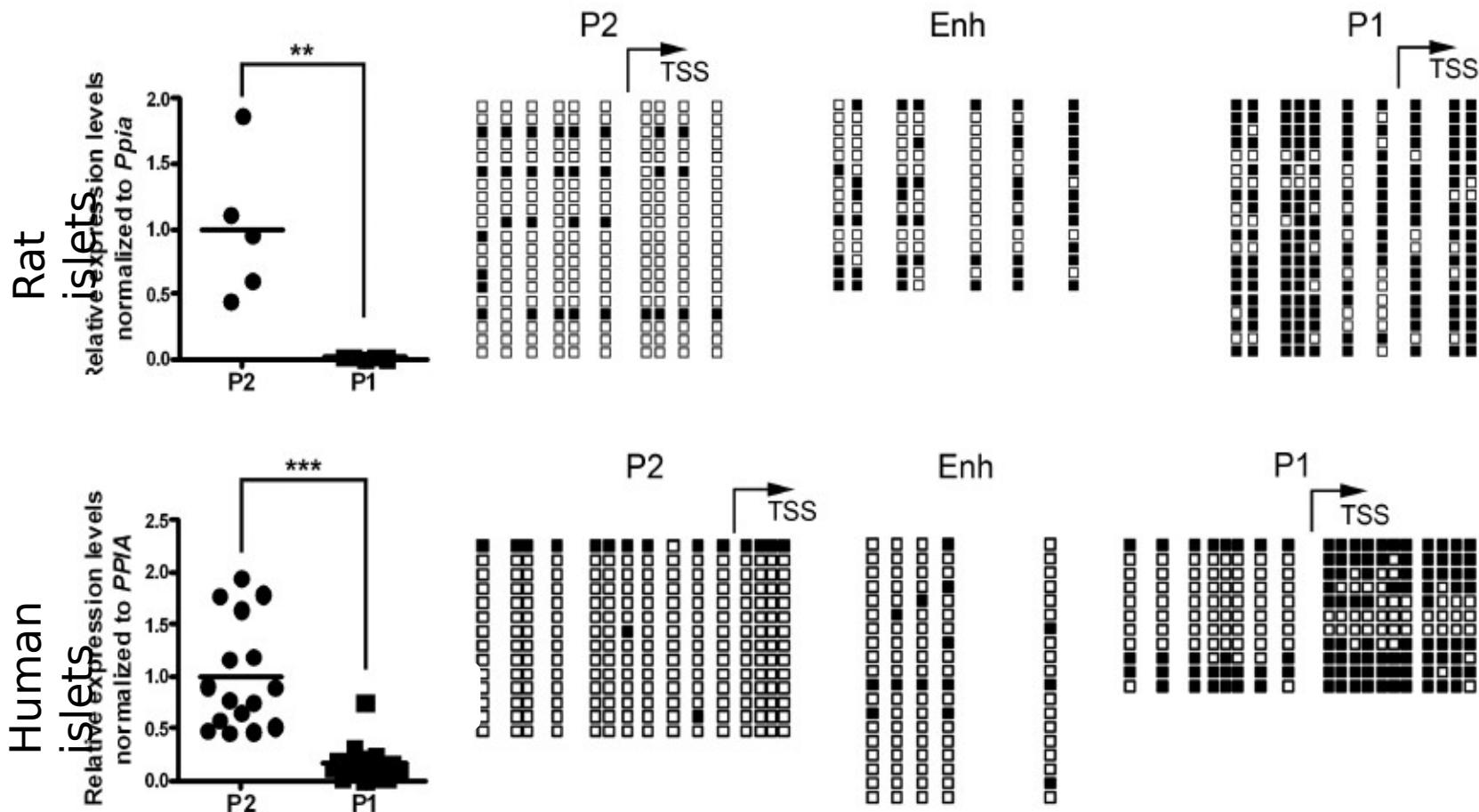


- Methylated CpG ○ Unmethylated CpG

- High-throughput Sequencing of DNA treated with bisulfite
- Other genome-wide methods include immunoprecipitation followed by arrays

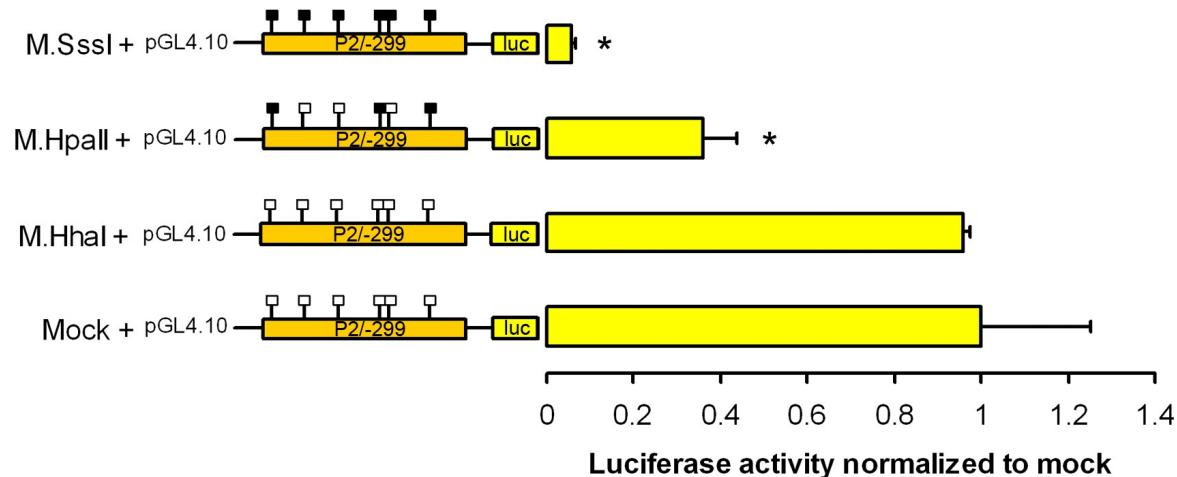
mCpG cause or effect of transcription silencing?

- ✓ Hnf4 α is a key developmental transcription factor from the nuclear receptor superfamily

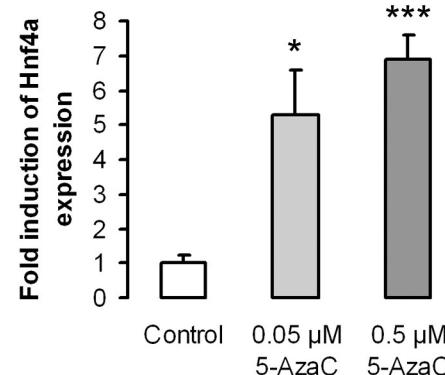
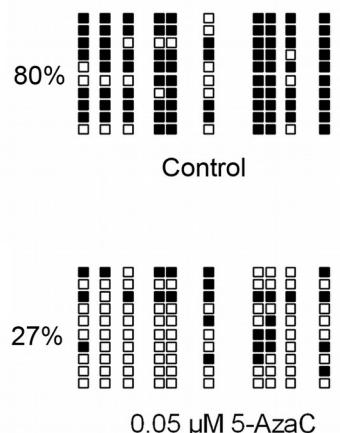


mCpG cause or effect of transcription silencing?

Methylation *in vitro* reduces promoter activity



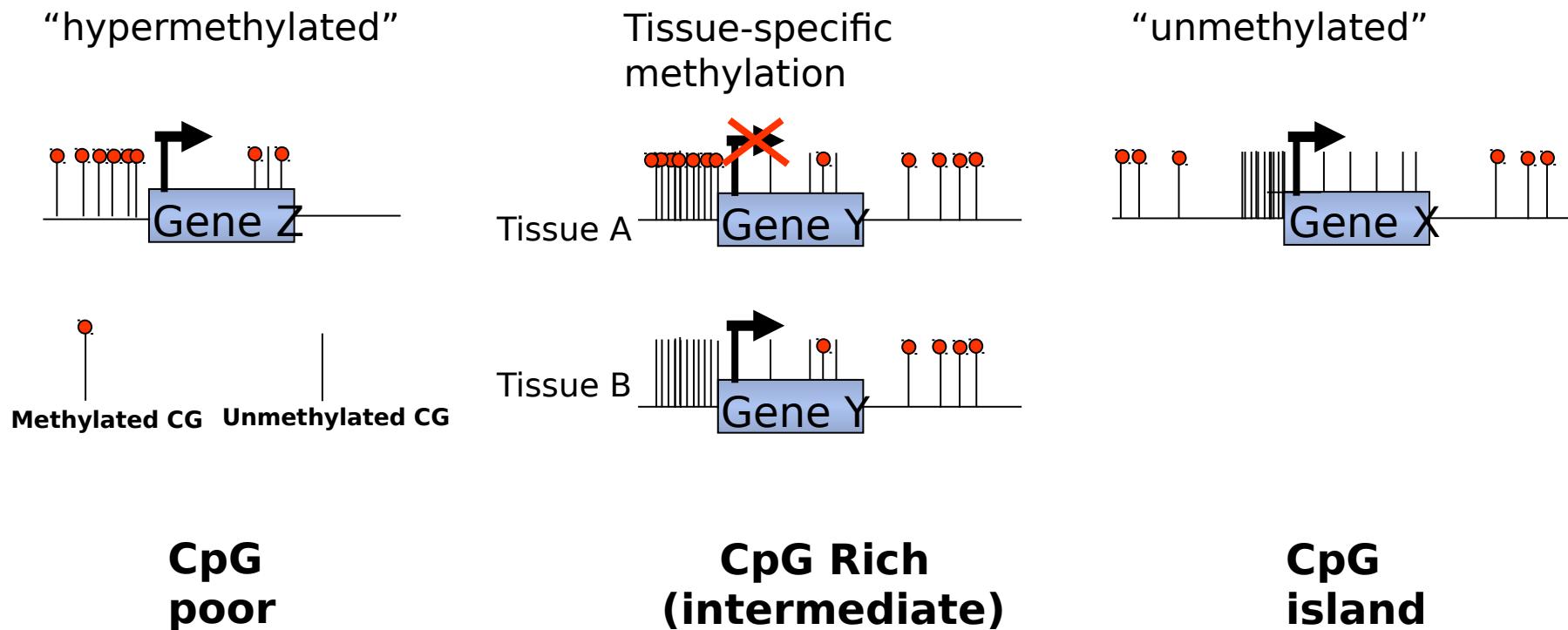
Demethylati
on
increases
expression



Genomic patterns of DNA methylation

- Most “stable” epigenetic mark
- Implicated in onset of disease
- Induced by the environment

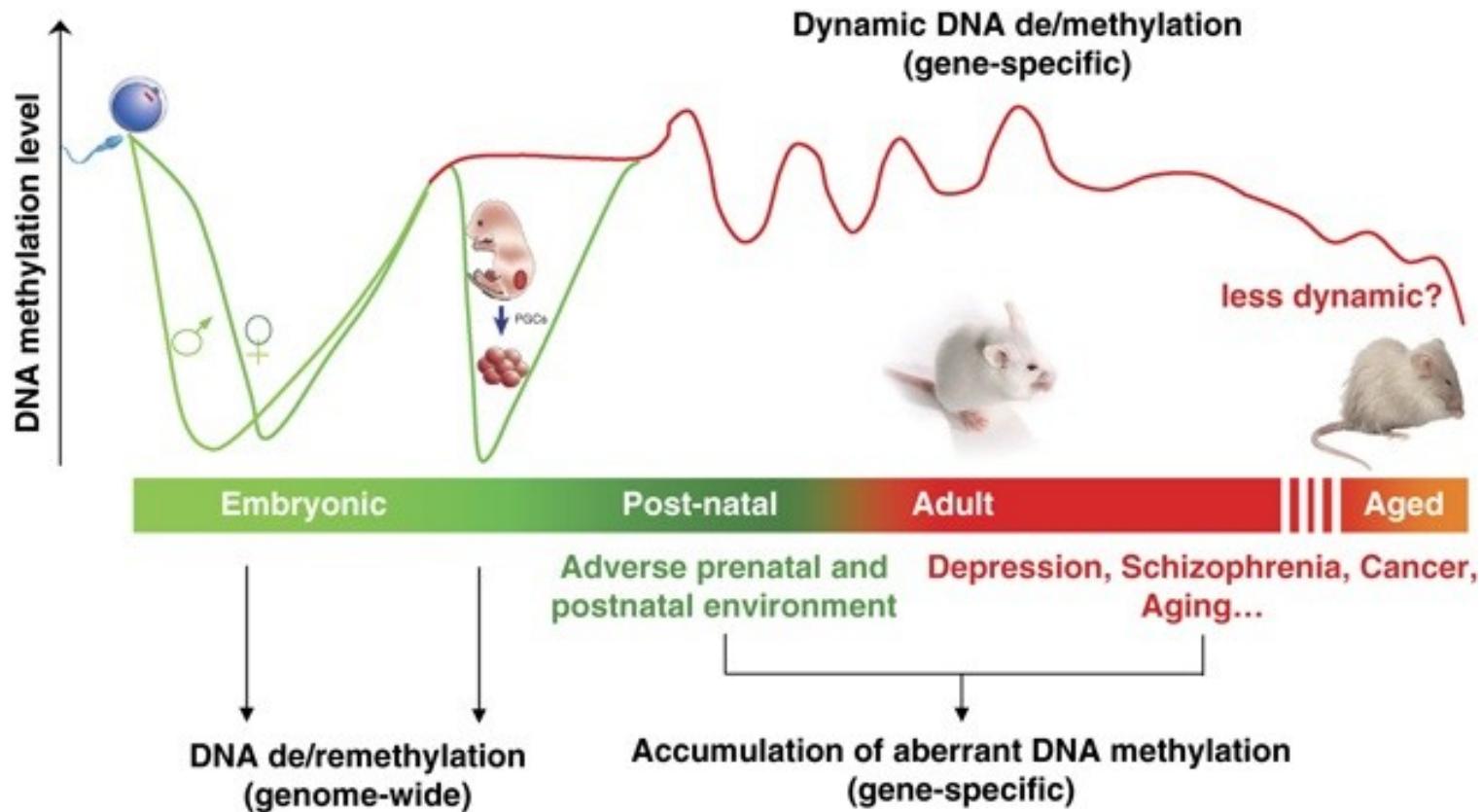
But: Does promoter methylation “always” correlate with gene silencing?



Conclusions

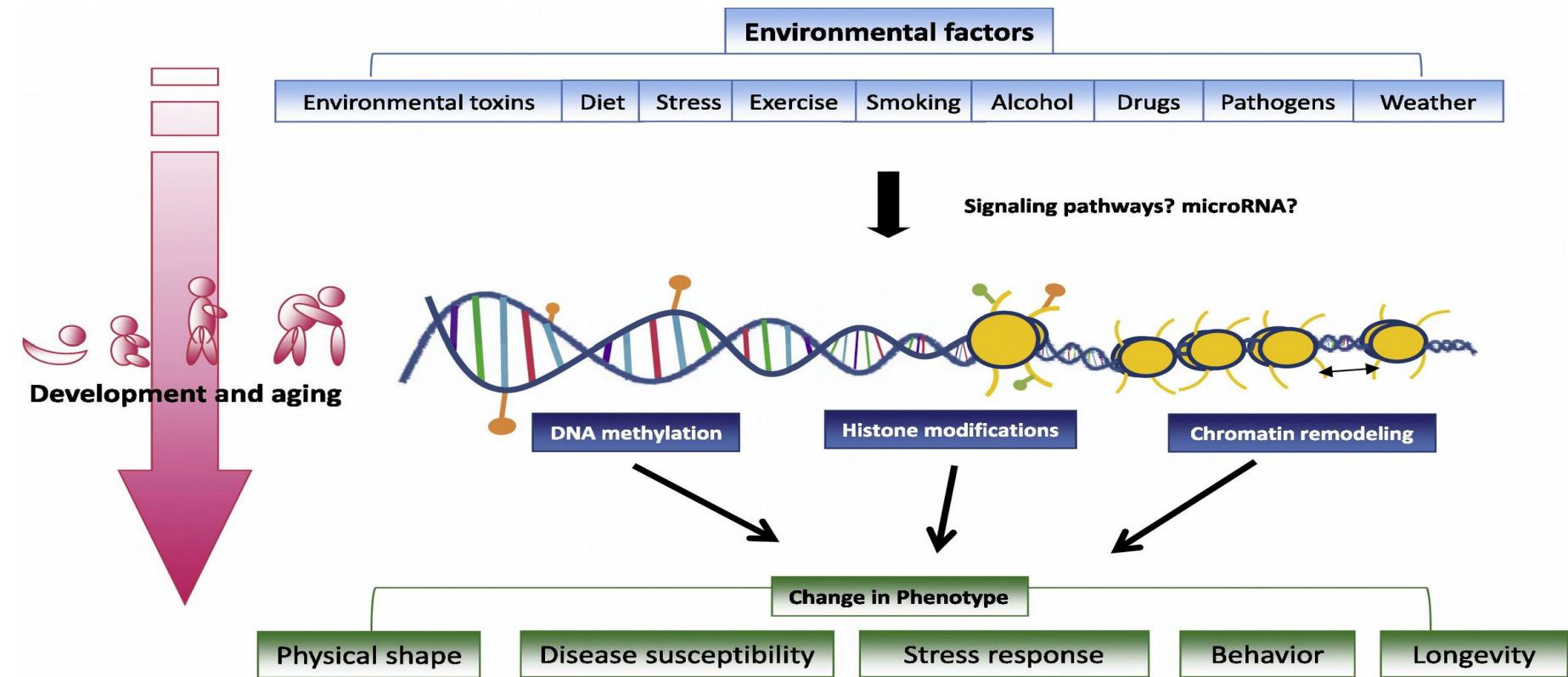
- CpG methylation is a mechanism of epigenetic memory
- Most CpGs in the vertebrate genome are methylated
- CpG islands are unmethylated, except for imprinted genes, X-inactivated genes, tumor-suppressor genes & key cell differentiation genes
- CpG patterns are stable in somatic cells but there is considerable dynamism during certain times in development
- Dnmt1 and Dnmt3-family proteins are responsible for establishing and maintaining global patterns of DNA methylation in mammals; active demethylation occurs by multiple mechanisms
- DNA methylation is associated with gene silencing
- Defects in DNA methylation are implicated in human disease
- Future challenges: how dynamic is DNA methylation? Is there a 'demethylase'? Role for nonCpG methylation? 5hmC, 5fc, 5caC? Other covalent DNA modifications?

Dynamics of epigenetic information during development



Somatic epigenetic patterns need to be ‘reset’ or ‘reprogrammed in early embryos and in germ cells in order to achieve developmental pluripotency

Environmental Epigenomic



Environmental Epigenomic

- Evidence that links epigenetics as potential mechanistic explanation for the long-term impact of the environment on physiology and behaviour:

Vernalization in plants



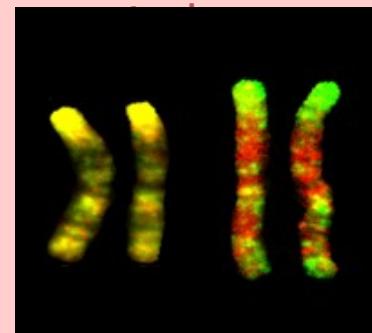
Epigenetic responses to cold temperatures & long-term memory

Royal Jelly – the ultimate in diet evolution



Nutritional control of reproductive status in honeybees via DNA methylation

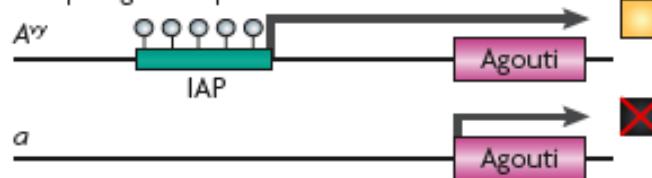
Epigenetic studies in Monozygous



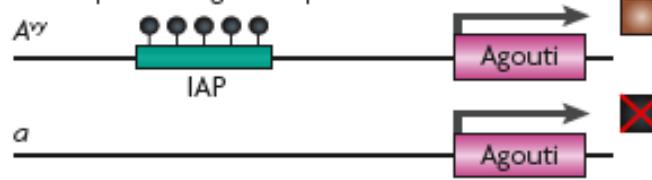
3 year-old 50 year-old
Divergent Disease susceptibility?

Maternal nutrition in Avy mice

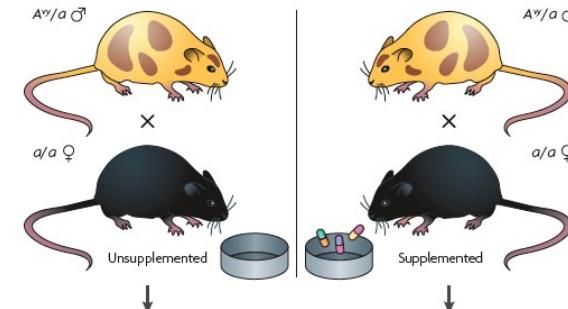
Ectopic agouti expression



Developmental agouti expression



a Dietary supplementation during pregnancy

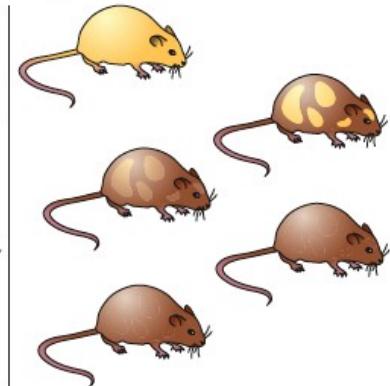


b A^y/a offspring

Unsupplemented mother

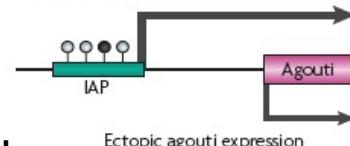


Supplemented mother

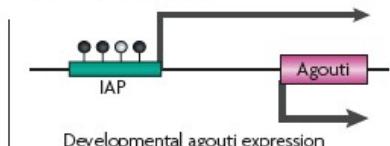


c Agouti expression

Unsupplemented mother



Supplemented mother

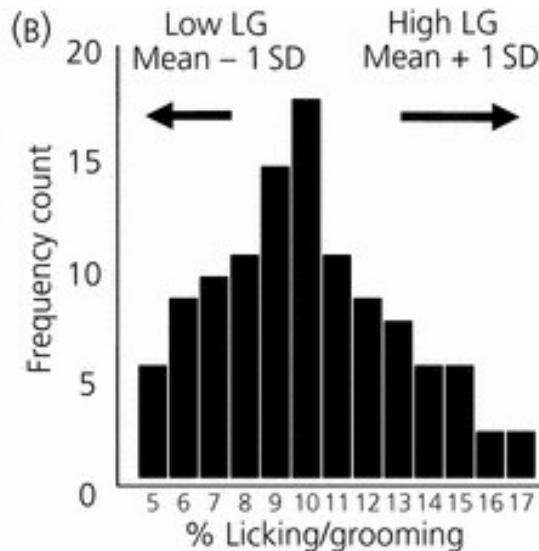


Effect of diet on DNA methylation and phenotype in offspring

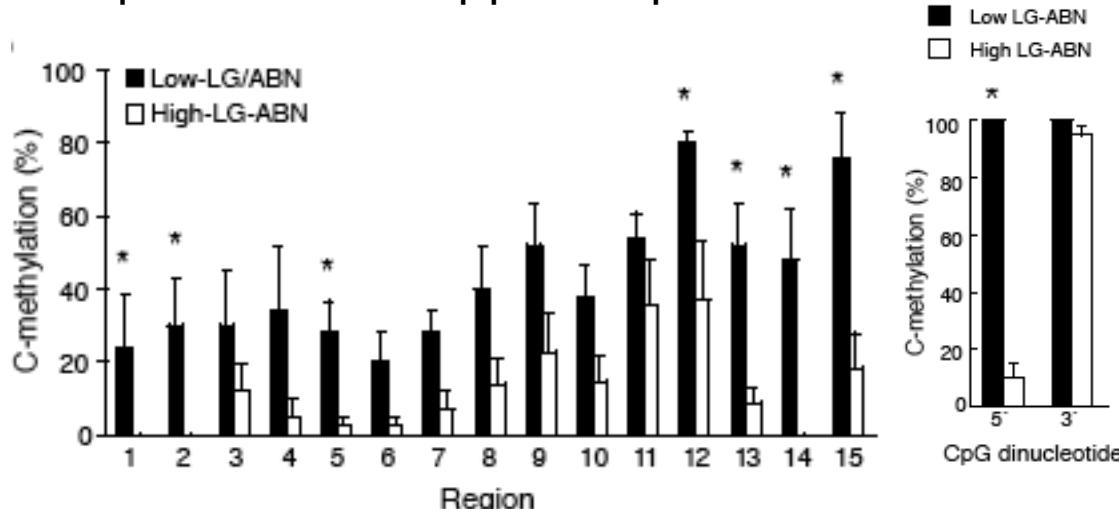
Waterland & Jirtle (2003) Mol Cell Biol 23:5293

Maternal behavior

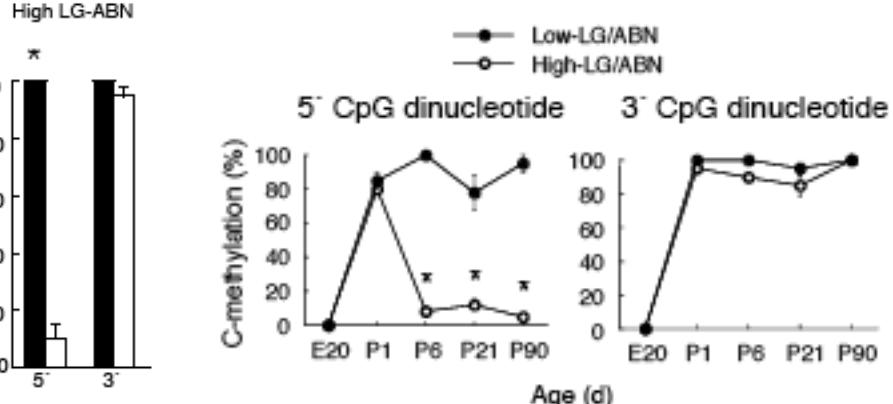
(A)



Maternal care alters cytosine methylation of GR promoter in hippocampus of offspring

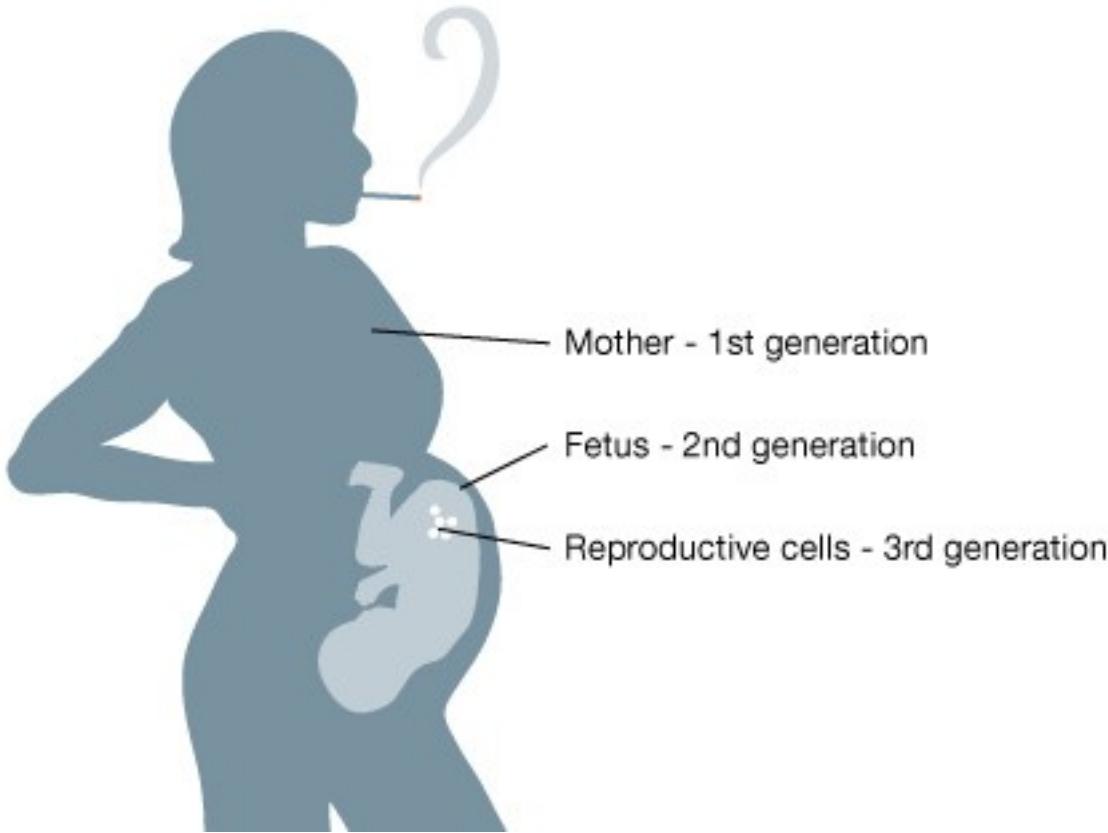


- Adult offspring of High LG are fearful & show modest HPA response to stress
- High LG show increased hippocampal GR expression and glucocorticoid sensitivity
- How does the effect of maternal care persist into adulthood?



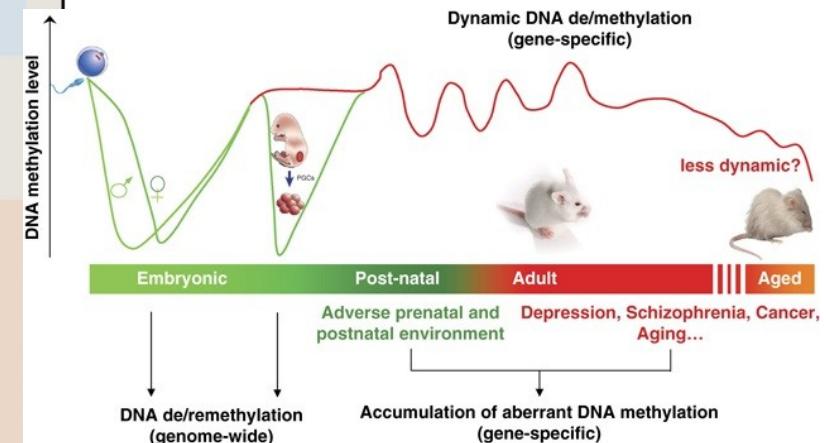
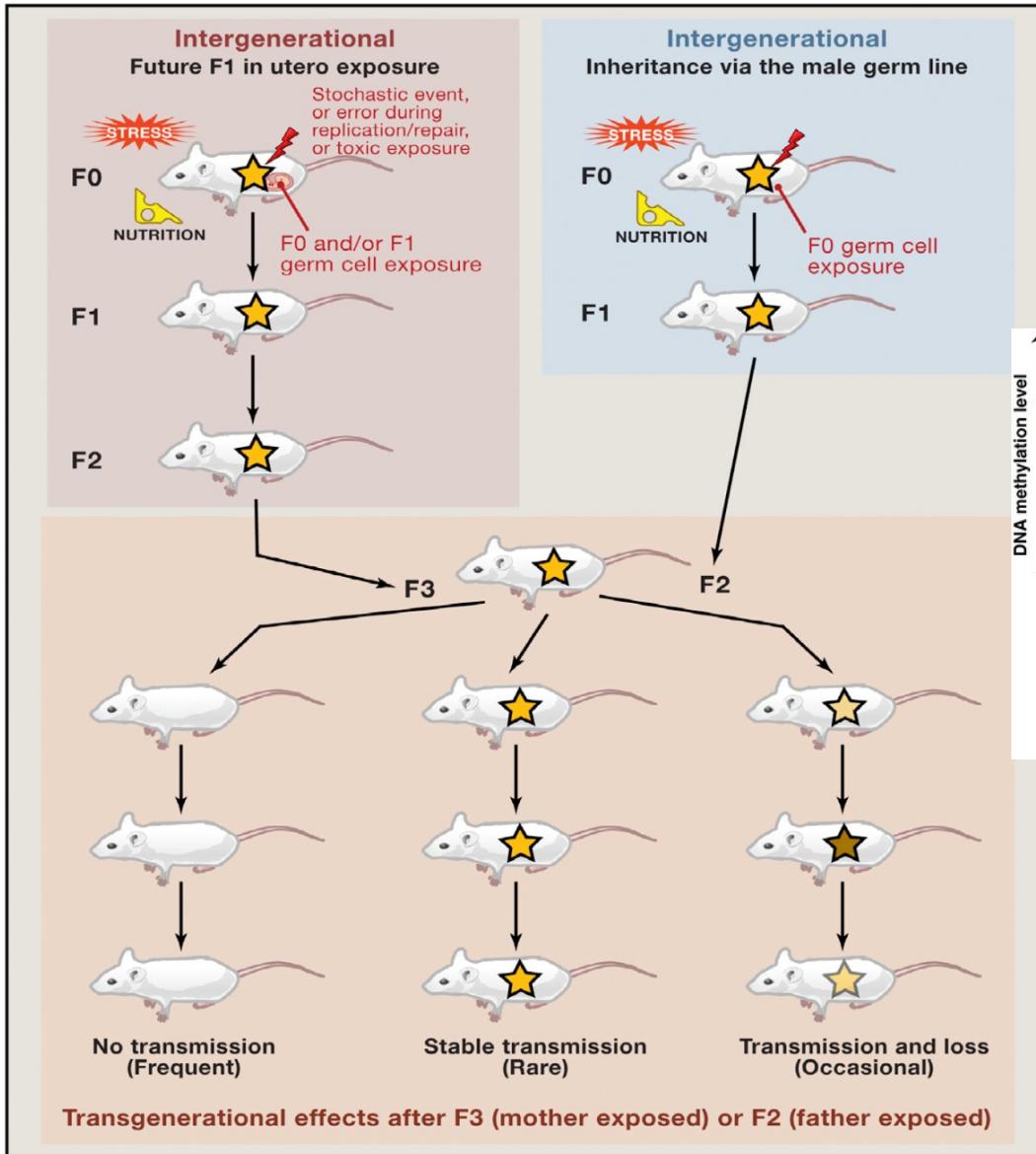
Weaver et al. (2004) *Nat Neurosci*

Epi genetic-induced generation changes



**- Rule out the possibility of genetic changes -
Show that the epigenetic effect can pass
through enough generations to rule out the
possibility of direct exposure**

Intergenerational vs. Transgenerational Epigenetic Inheritance



Paternally Induced Transgenerational Environmental Reprogramming of Metabolic Gene Expression in Mammals

Benjamin R. Carone,^{1,10} Lucas Fauquier,^{1,10} Naomi Habib,^{4,5,10} Jeremy M. Shea,^{1,10} Caroline E. Hart,¹ Ruowang Li,² Christoph Bock,^{6,7} Chengjian Li,¹ Hongcang Gu,⁶ Phillip D. Zamore,^{1,3} Alexander Meissner,^{6,7} Zhiping Weng,² Hans A. Hofmann,⁸ Nir Friedman,^{4,9} and Oliver J. Rando^{1,*}

LETTER

doi:10.1038/nature09491

Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring

Sheau-Fang Ng¹, Ruby C. Y. Lin², D. Ross Laybutt³, Romain Barres⁴, Julie A. Owens⁵ & Margaret J. Morris¹

Insights into genomic imprinting from mouse models



Miguel Constâncio

Metabolic Research Laboratories, Department of Obstetrics & Gynaecology
University of Cambridge; email: jmasmc2@cam.ac.uk

Outline

- How do mouse models contribute to our understanding of genomic imprinting?

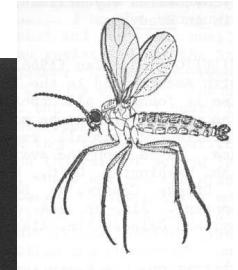
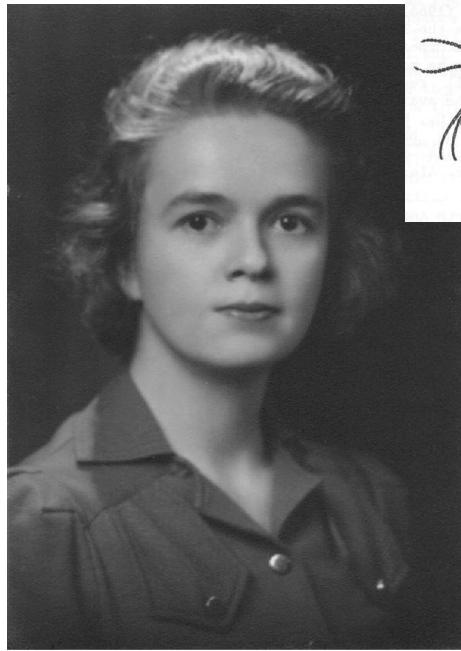
Part 1. The discovery of imprinting & imprinted genes

Part 2. Deciphering the molecular basis of imprinting

Part 3. The function of imprinting

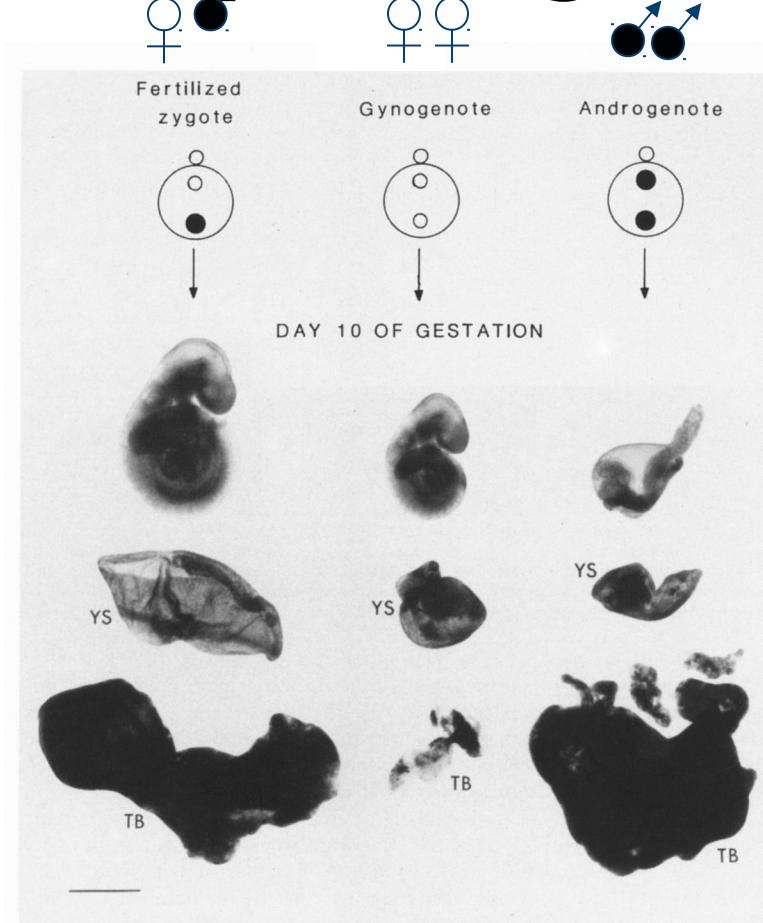
Marking parental origin of genes

- Concept of a marking or imprinting of chromosomes was first introduced by Helen Crouse (1960) who proposed “chromosome imprinting” to explain the regulated loss of paternal X-chromosomes during development of *Sciara* embryos
- In mammals, the term genomic imprinting was first applied to the preferential inactivation of the paternal X chromosome in extra-embryonic lineages in the mouse
(Takagi and Sasaki, 1975)



Gerbi S A Genetics 2007;175:1-6

- The embryological evidence for imprinting:

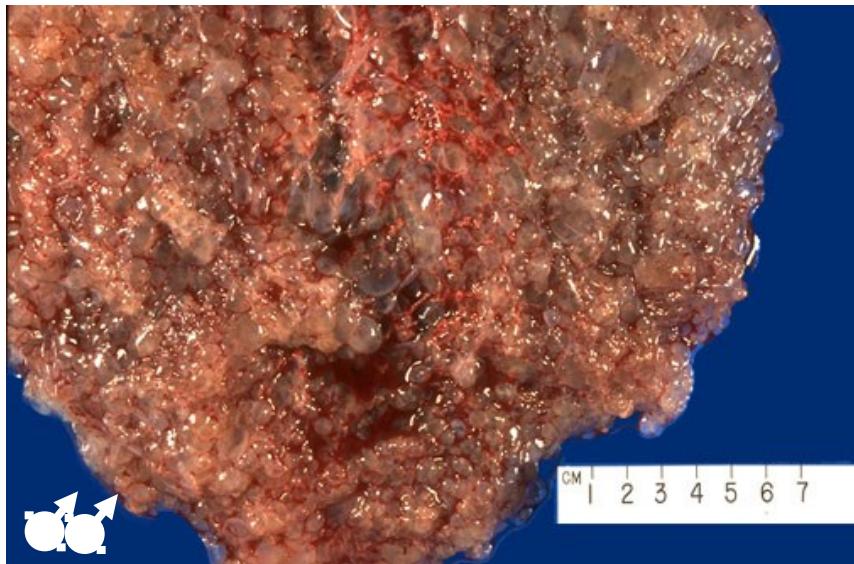


“....This suggests of the existence of genes that are only expressed from one parental genome or the other...”

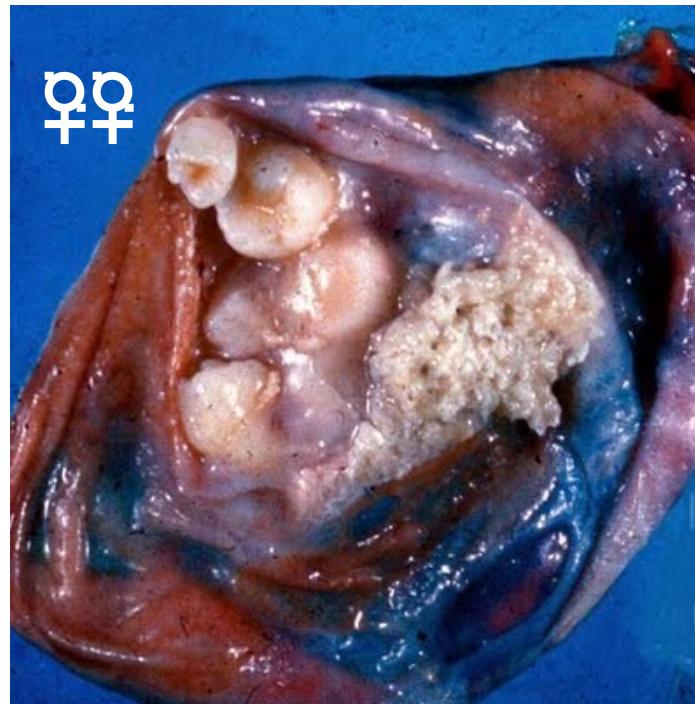
Surani et al (1984) *Nature* **308**:548-550

McGrath & Solter (1984) *Cell* **37**:179

Human androgenetic and gynogenetic embryos

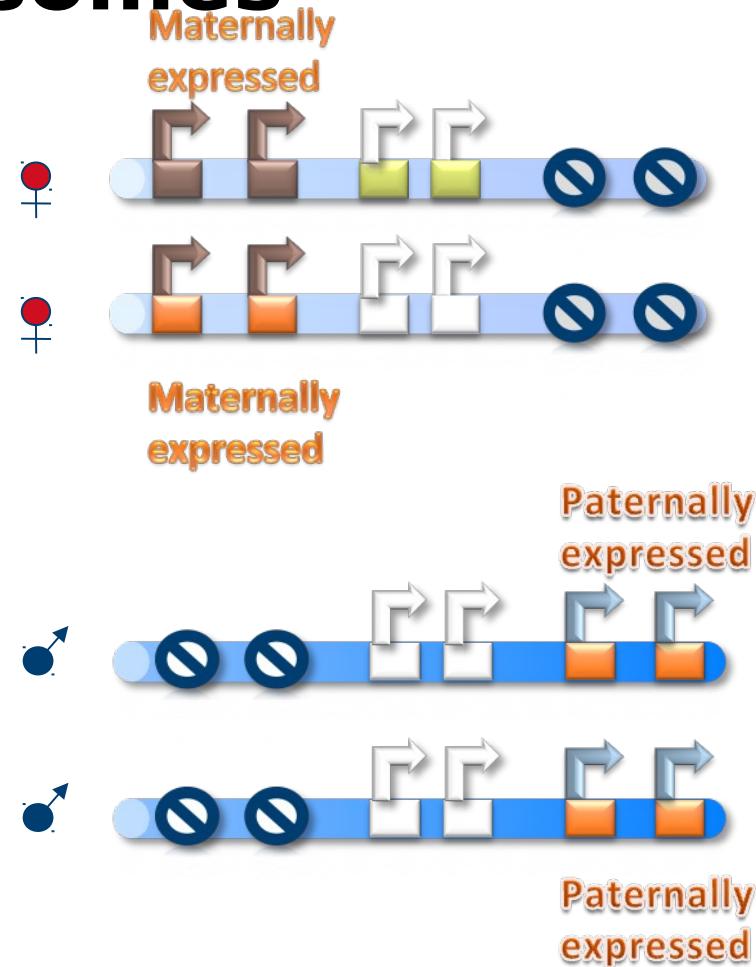
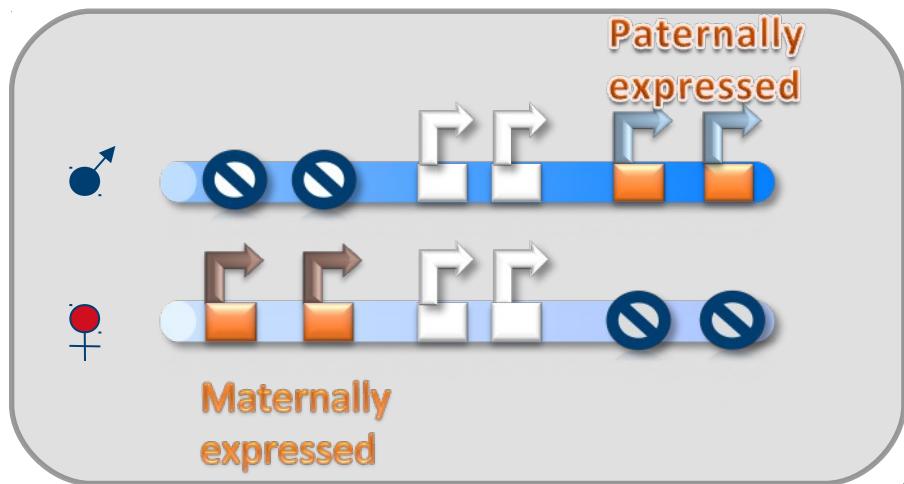


Complete hydatidiform mole

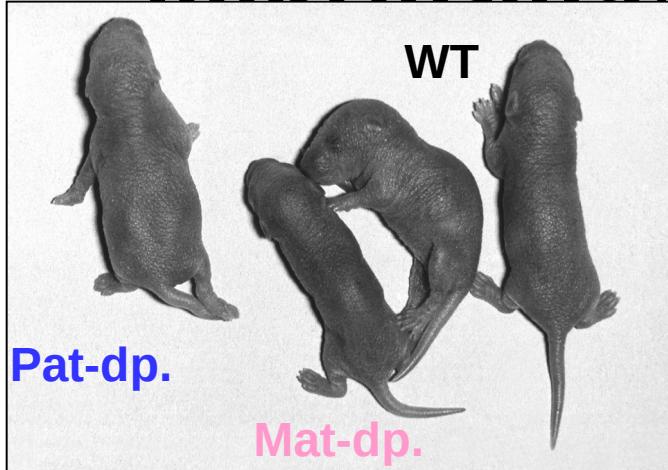


Ovarian teratoma

Imprinted genes are expressed from one of the parental chromosomes



- The genetic evidence for imprinting:



Uni-parental disomies of distal *mChr2*

- Mice with a paternal duplication (PatDp) have broad flat backs and oedema; hyperkinetic
- Mice with a maternal duplication (MatDp) have narrow bodies and fail to suckle; hypokinetic

Cattanach and Kirk, Nature (1985) 315:496-498

- Mice that inherited specific chromosomes from one parent only showed developmental defects, indicating the presence of imprinted genes on the disomic segment
- Genomic regions that might harbour imprinted genes were mapped by analysing uniparental

Parental genomes are not functionally equivalent



Imbalance of parental contribution and human disease

15q11-13:

- Angelman syndrome – lack of maternal contribution
- Prader-Willi syndrome – lack of paternal contribution

Angelman and Prader-Willi syndromes

Finding the first imprinted genes

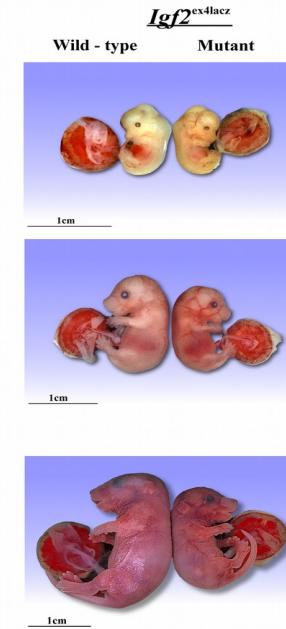
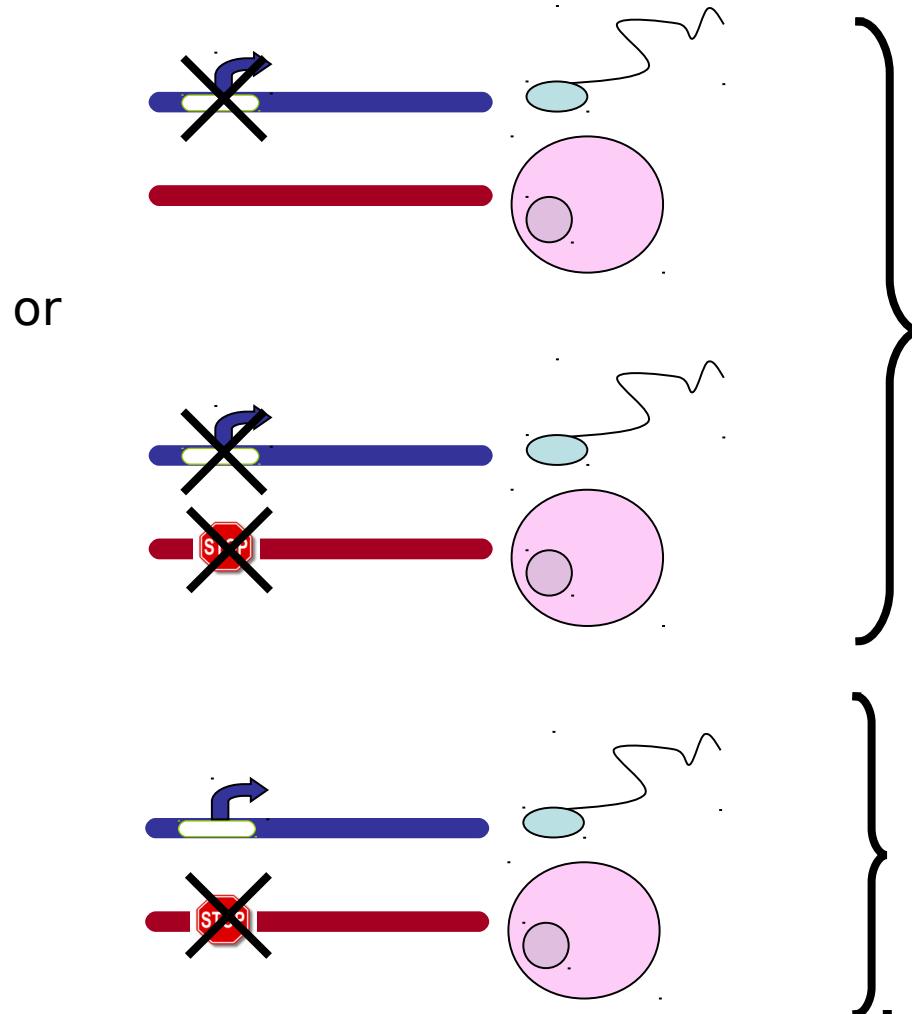
- *H19*, *Igf2r* and *Igf2* were the first imprinted genes to be discovered (all in 1991)

H19 by allele-specific gene expression

Igf2r by chromosomal deletion (Tme locus)

Igf2 by gene knock-out

Igf2 imprinting discovery by gene knock-out



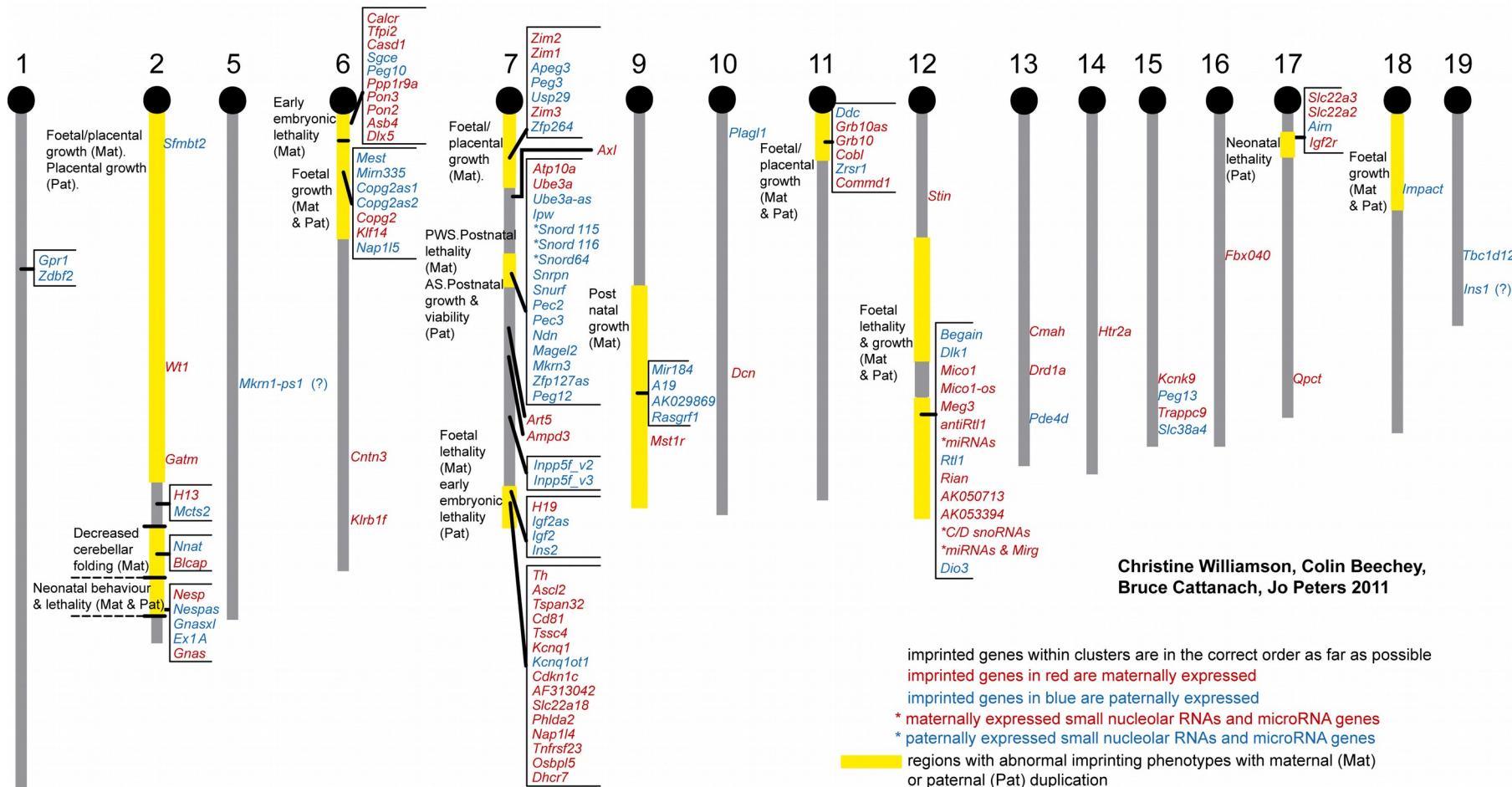
Normal birth weight

Igf2 is a paternally expressed gene DeChiara *et al.* (1991) Cell 66

The Imprinted

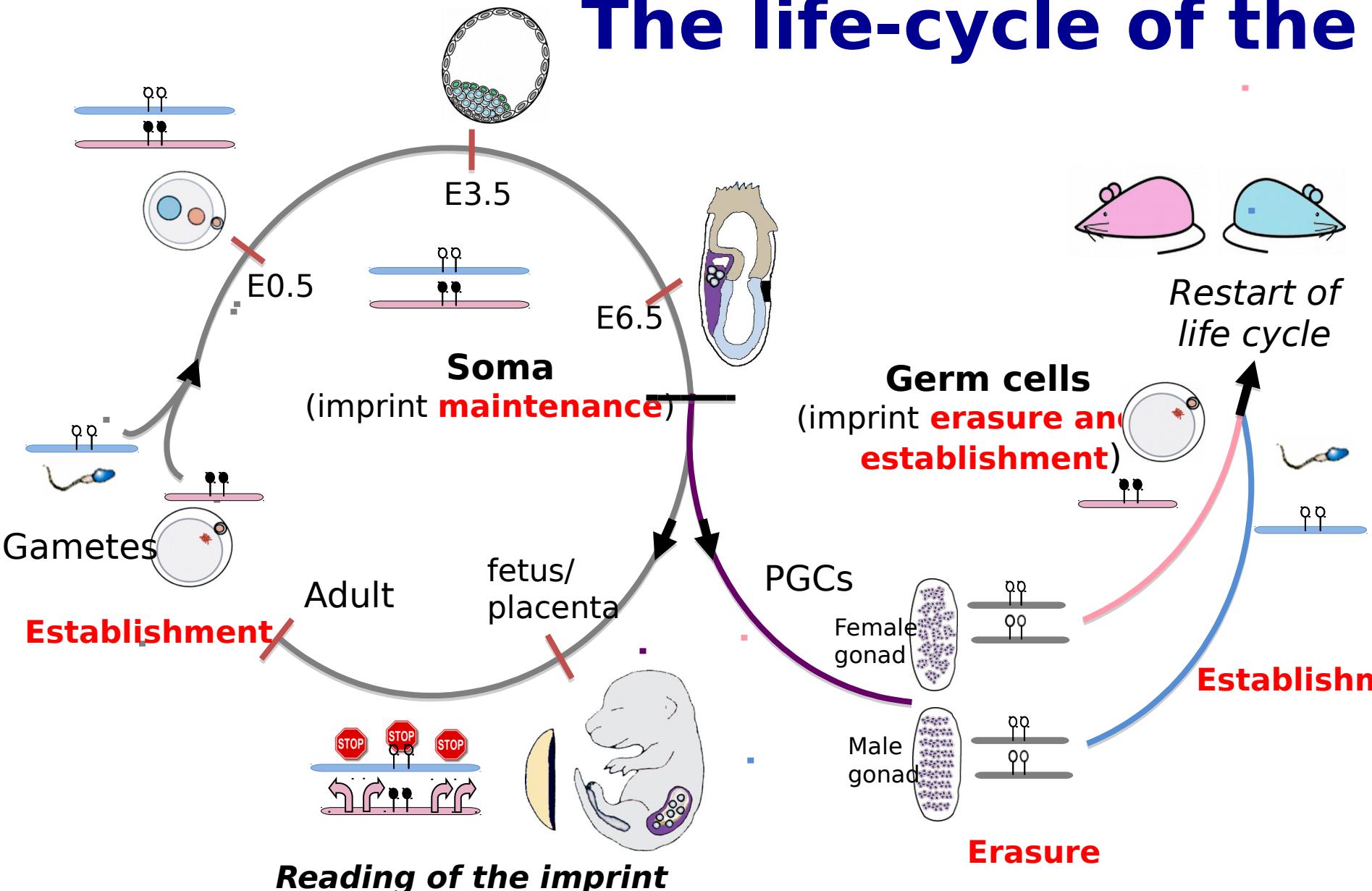
Mouse Imprinted Genes, Regions and Phenotypes

Chromosome:



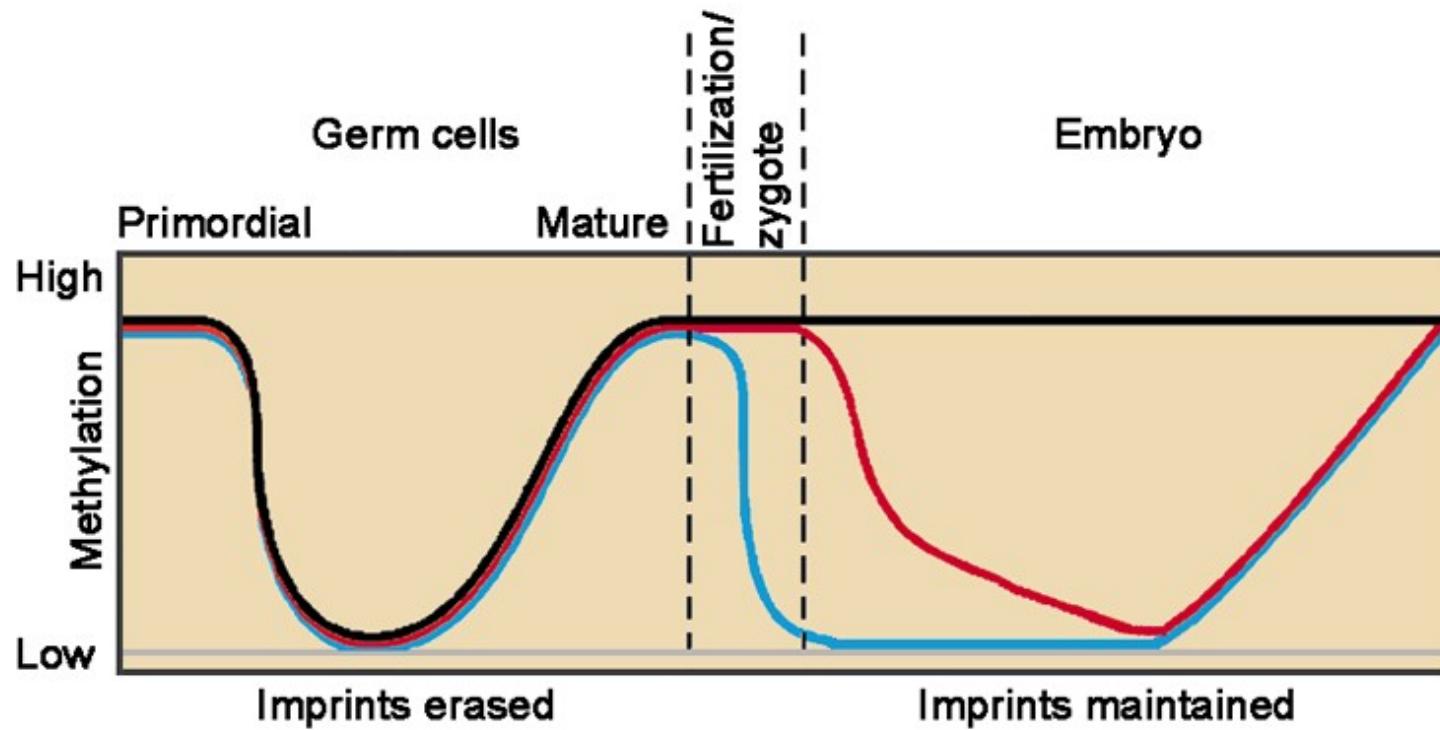
Christine Williamson, Colin Beechey,
 Bruce Cattanach, Jo Peters 2011

The life-cycle of the i



Unmethylated ICR
Methylated ICR

Imprinted genes resist epigenetic reprogramming in early embryos

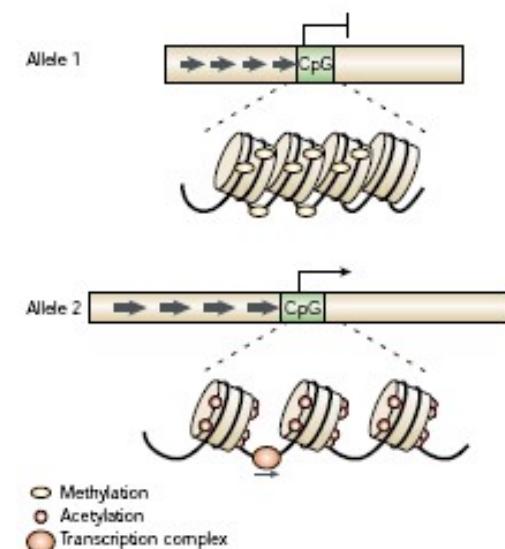


Imprinting marks carried in the egg and sperm survive epigenetic re-programming events following fertilization when the male and female genome meet up

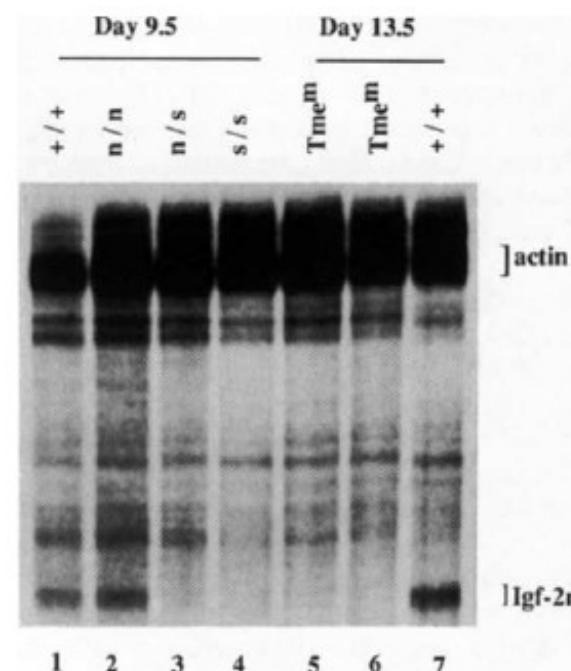
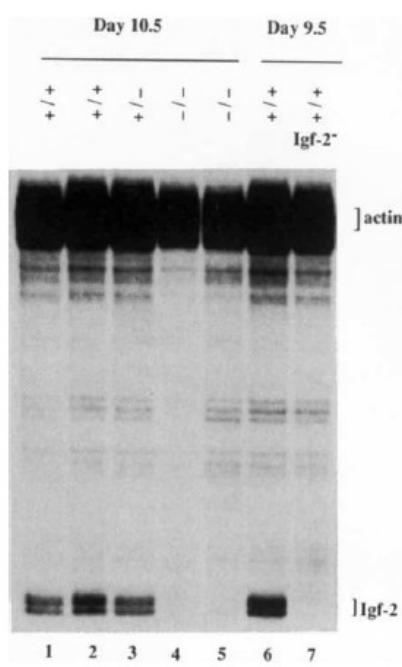
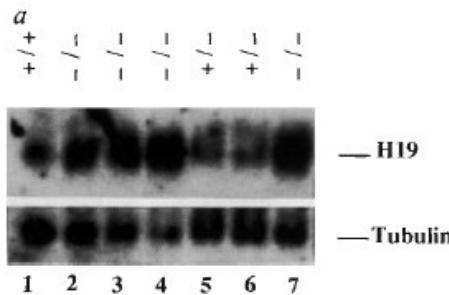
Role for DNA methylation in genomic imprinting

En Li*, Caroline Beard & Rudolf Jaenisch†

Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA

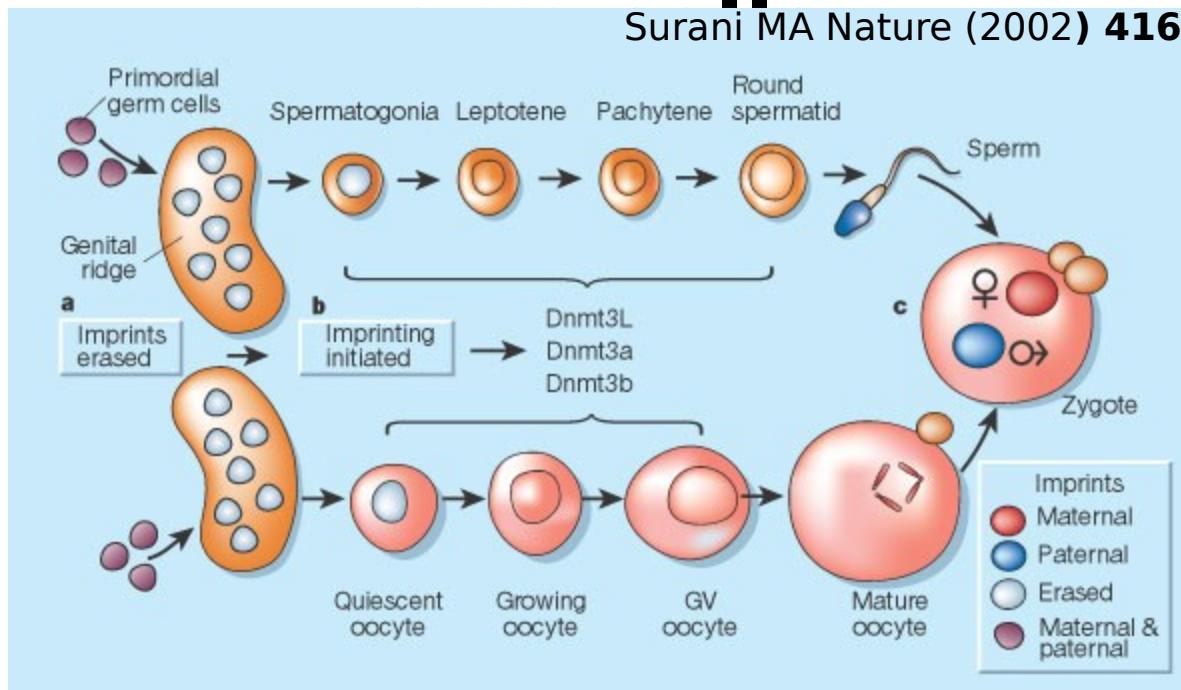


Reik & Walter (2001) Nat Rev Genet 2



Dnmt3a and Dnmt3L are required for methylation of most imprinted loci in

Surani MA Nature (2002) 416:491-493



letters to nature

REPORTS

Essential role for *de novo* DNA methyltransferase Dnmt3a in paternal and maternal imprinting

Masahiro Kaneda^{1,2}, Masaki Okano^{3,4}, Kenichiro Hata¹, Takashi Sado^{1,5,6}, Naomi Tsujimoto^{1,2,4*}, En Li^{4*} & Hiroyuki Sasaki^{1,2}

Nature (2004) 429:900-903

Dnmt3L and the Establishment of Maternal Genomic Imprints

Déborah Bourc'his,¹ Guo-Liang Xu,^{1,*} Chyuan-Sheng Lin,² Brooke Bollman,^{1,†} Timothy H. Bestor^{1,‡}

Science (2001) 294:2536-2

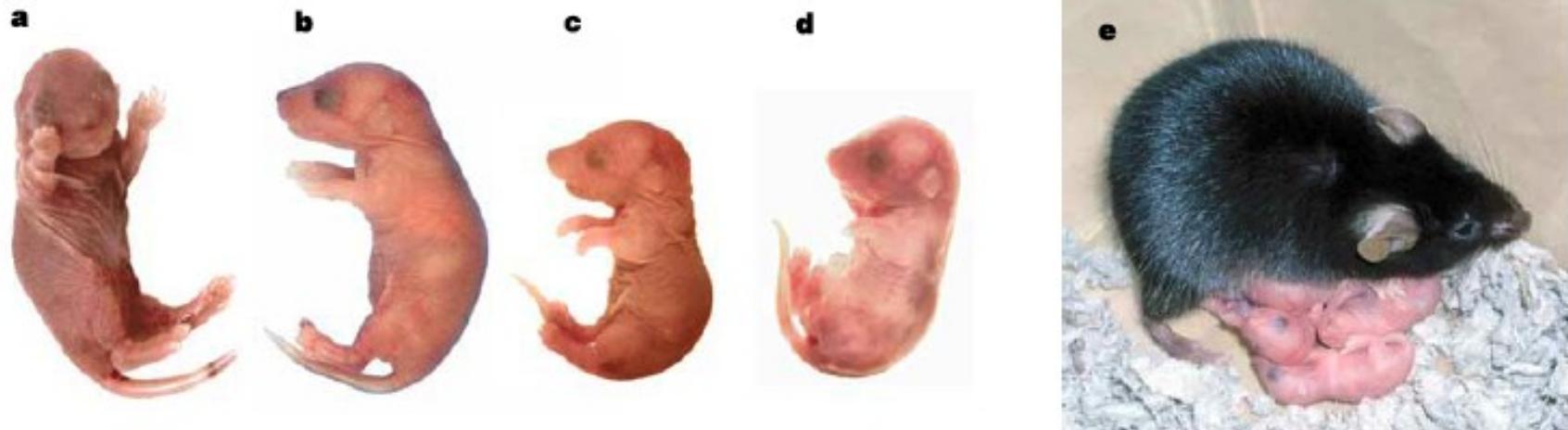
Birth of parthenogenetic mice that can develop to adulthood

**Tomohiro Kono^{1,3}, Yayoi Obata^{1,3}, Quiong Wu^{1,3}, Katsutoshi Niwa^{1,3},
Yukiko Ono¹, Yuji Yamamoto^{2,3}, Eun Sung Park⁴, Jeong-Sun Seo^{4,5}
& Hidehiko Ogawa^{1,3}**

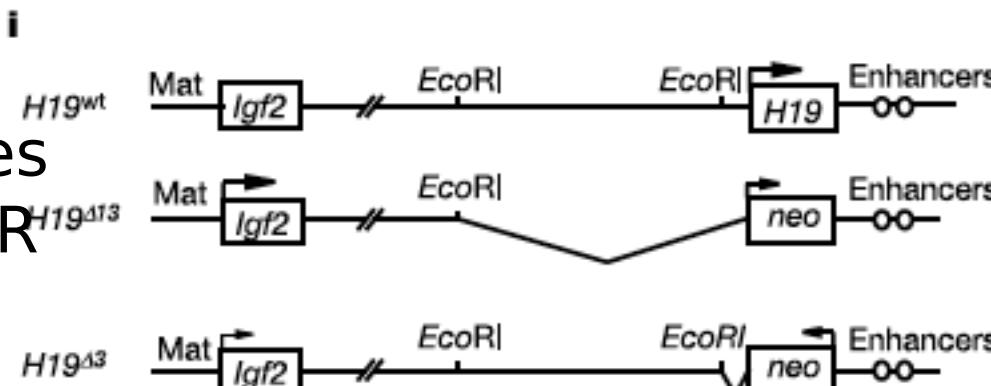
- Mouse parthenogenetic embryos die by E10 of gestation
- Parthenogenetic mouse embryos that contain genomes from non-growing (ng) and fully grown (oocytes) can develop to E13.5 by appropriate expression of many imprinted genes

“It is possible to obtain a viable adult mouse from two maternal genomes”

Kaguya



Appropriate expression of Igf2 and H19



“Ng” oocytes
with H19 ICR
deletion

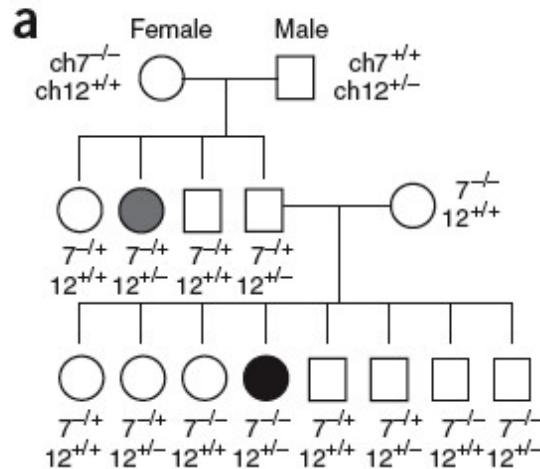
Birth (2/371)

E17.5

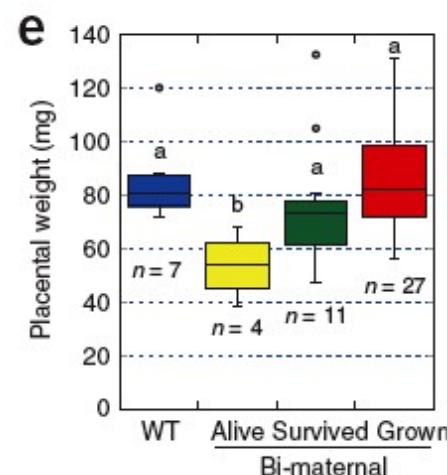
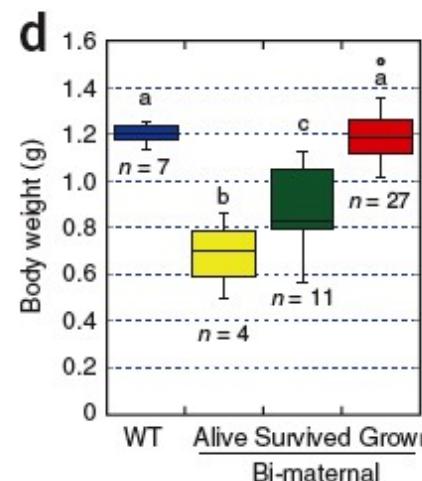
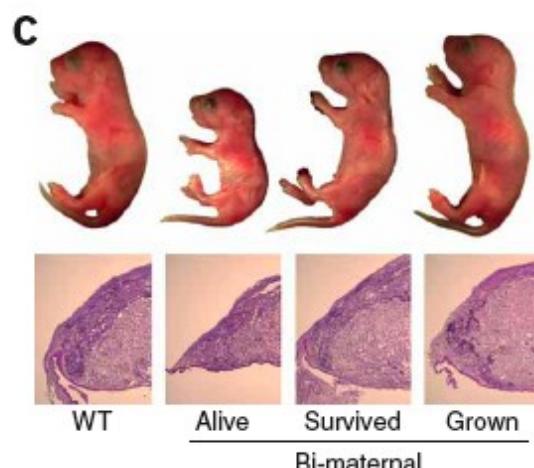
Nature (2004) 428: 860

High-frequency generation of viable mice from engineered bi-maternal embryos

Manabu Kawahara^{1,2}, Qiong Wu^{1,2,4}, Nozomi Takahashi¹, Shinnosuke Morita¹, Kaori Yamada¹, Mitsuteru Ito³, Anne C Ferguson-Smith³ & Tomohiro Kono^{1,2}



Appropriate expression of Igf2 and H19 and Dlk-Gtl2



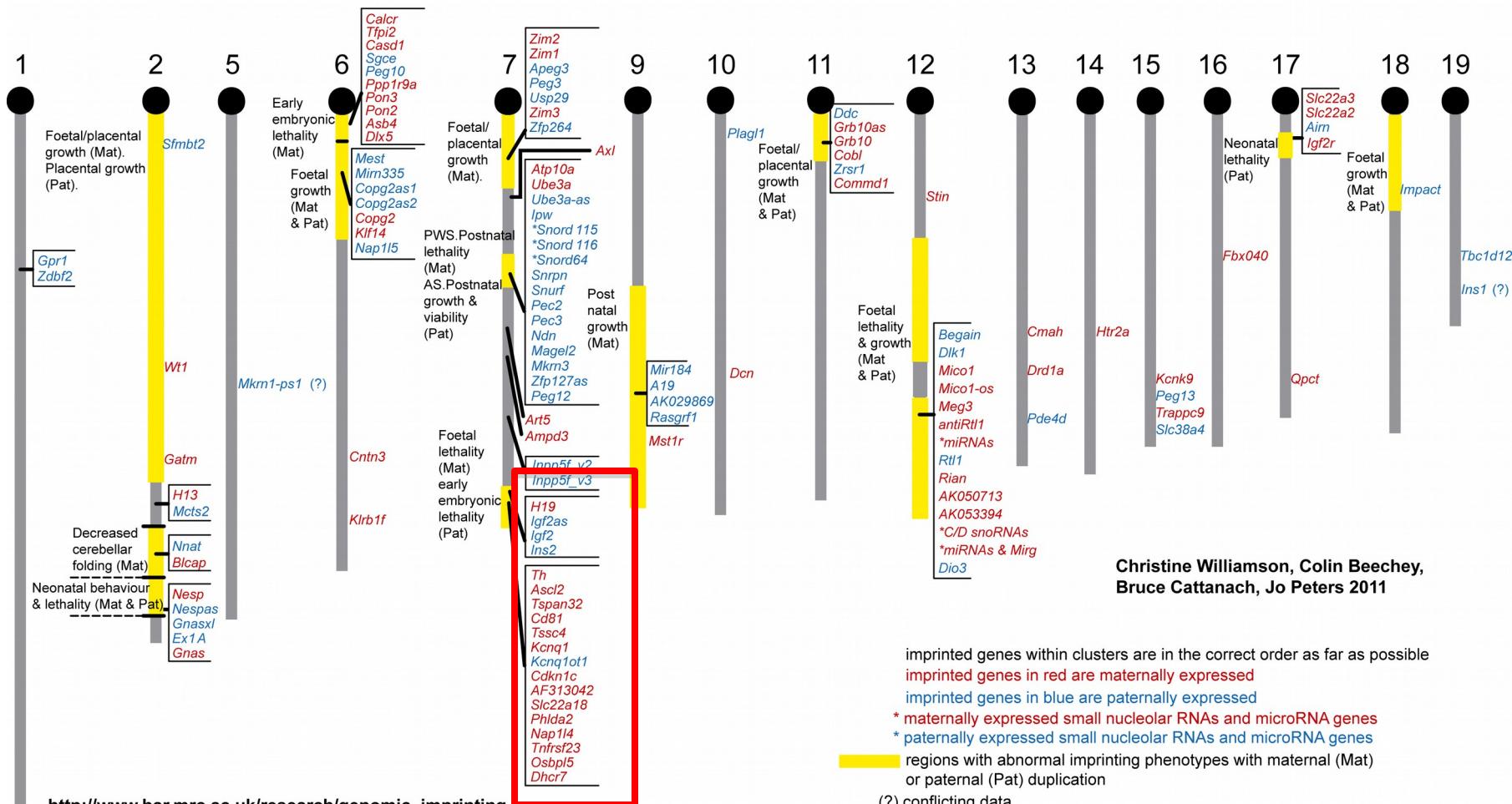
Summary of Part 1. The discovery of imprinting & imprinted genes

- The embryological and genetic evidence for imprinting:
 - nuclear transplantation experiments
 - uniparental disomies
- Finding imprinted genes:
 - allele-specific expression (candidate/genome-wide)
 - knock-outs/large deletions
- Generating viable mice without a father

The Imprinted

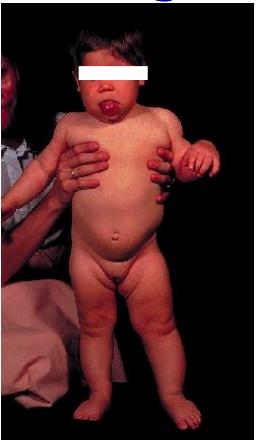
Mouse Imprinted Genes, Regions and Phenotypes

Chromosome:



Distal chromosome 7 and 11p15.5 imprinting clusters play major roles in fetal growth

- Genes involved in fetal growth and placenta development
- Involved in fetal growth Syndromes:

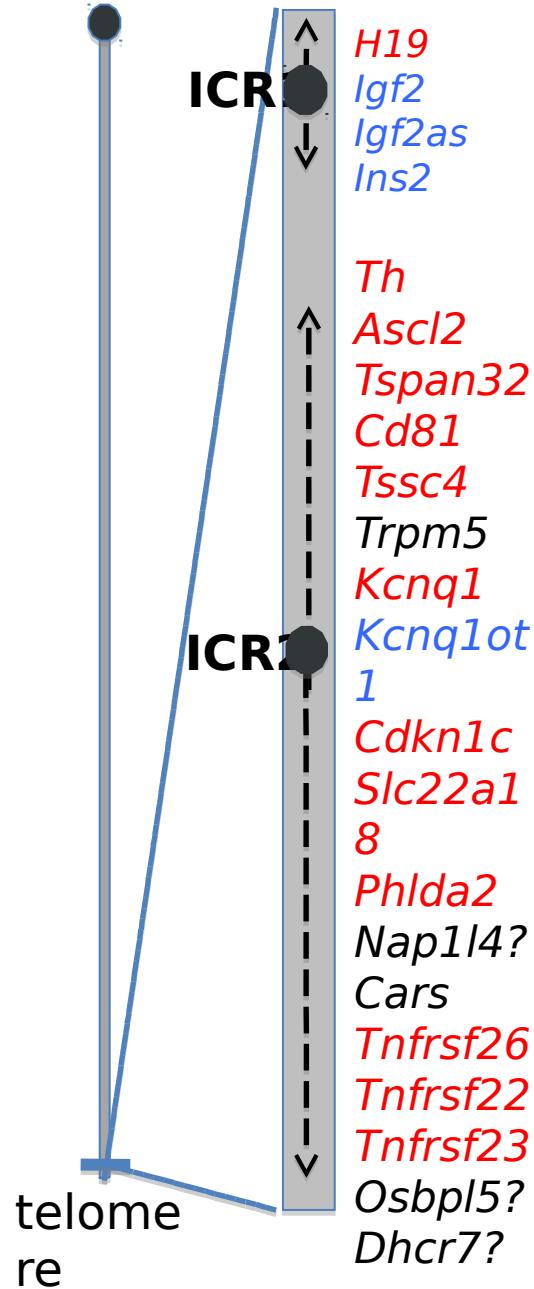


Beckwith-Wiedemann
Overgrowth

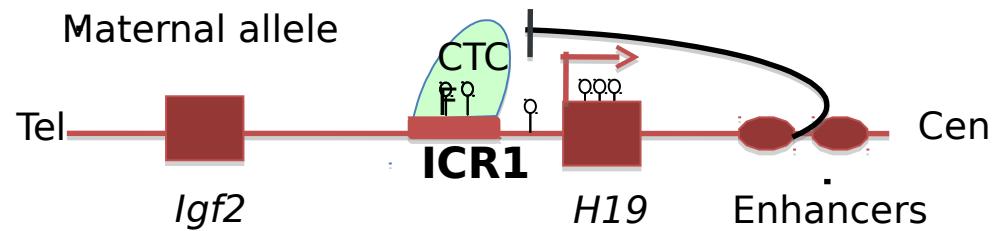
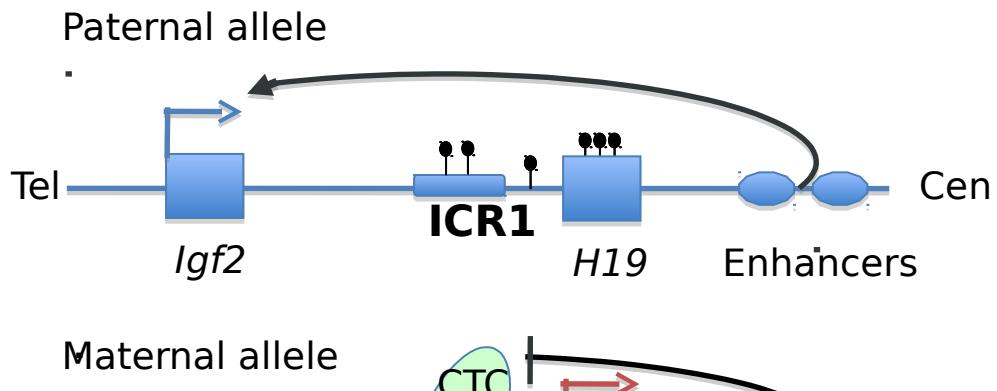


Silver-Russell
FGR

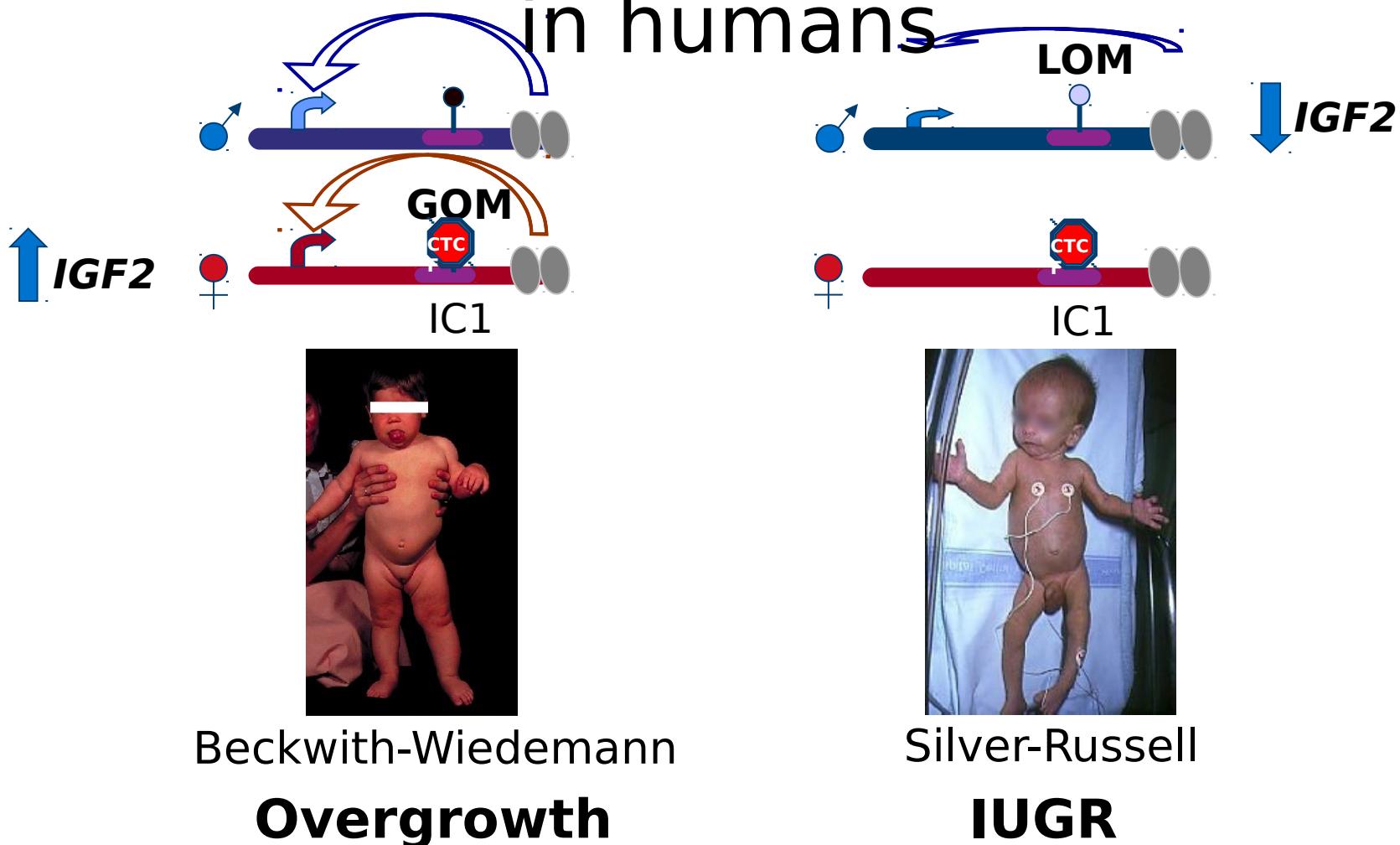
centromere



ICR1- Regulation of an imprinting cluster through a CTCF-dependent boundary



IGF2 is a major determinant of fetal growth in humans



Beckwith-Wiedemann

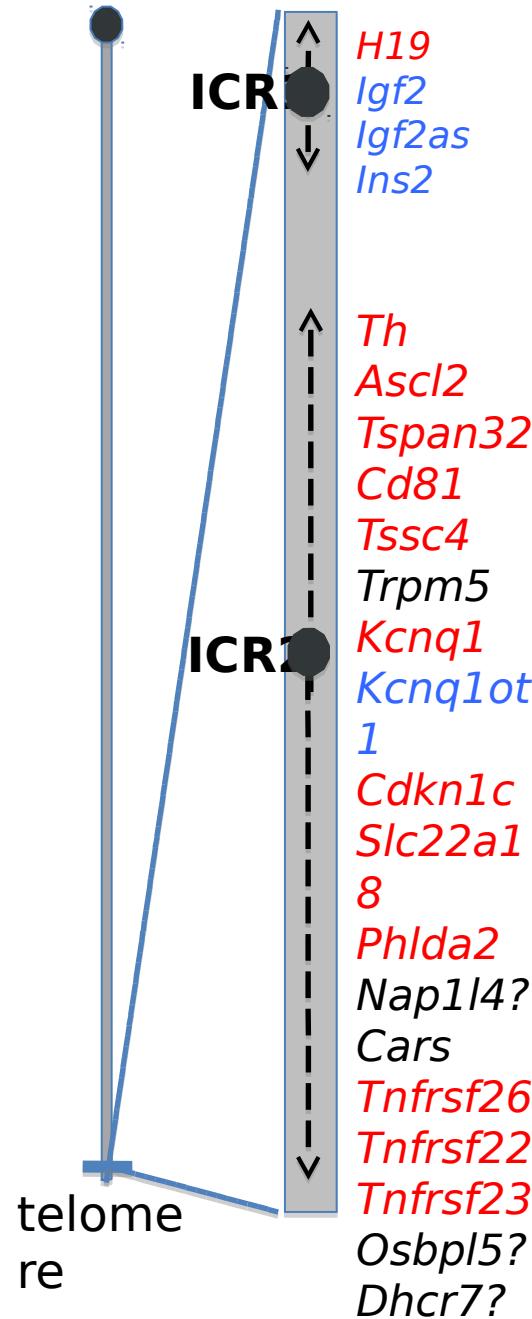
Overgrowth

Silver-Russell

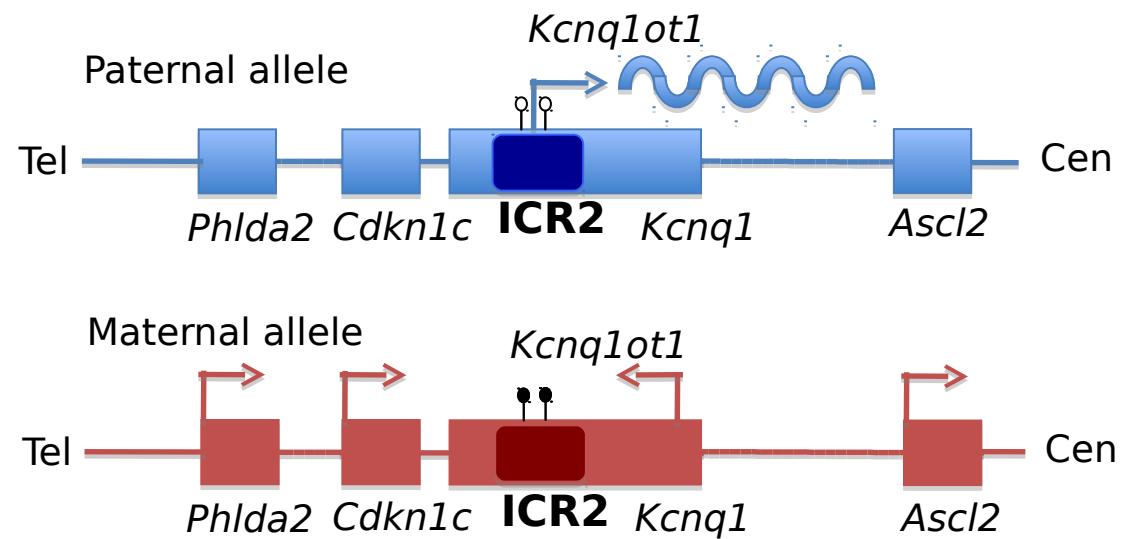
IUGR

Cerrato F et al. (2008) *Hum Mol Genet* **17**:1427
Gicquel C et al. (2005) *Nat Genet*

centromere



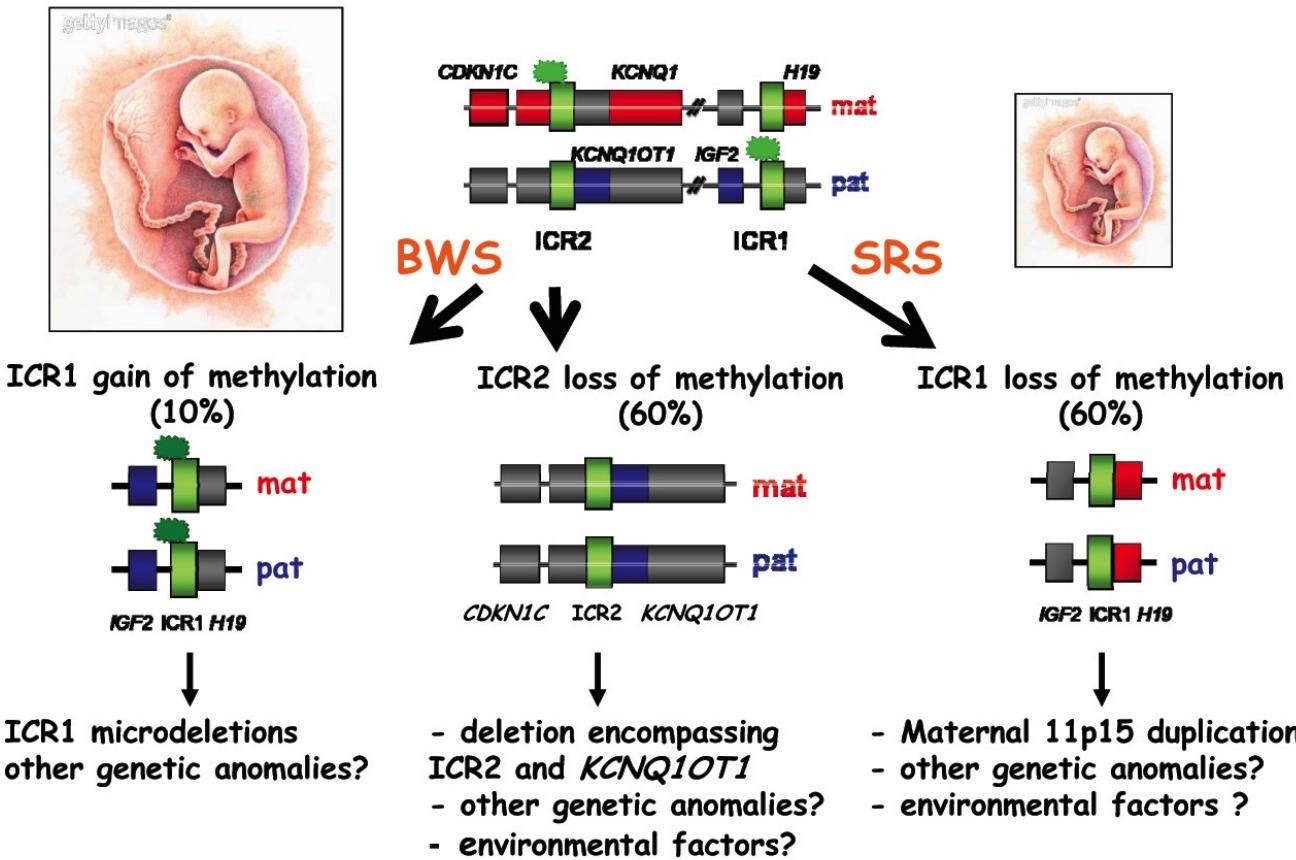
ICR2- Regulation of an imprinting cluster through a non-coding RNA



Ideraabdullahah et al. (2008) *Mutat Res* **647**:7; Edwards & Ferguson-Smith (2007) *Curr Opin Cell Biol* **19**:281; Lewis & Reik (2006) *Cytogenet Genome Res* **113**: 81

Epigenetic regulation of ICR1/ICR2 and BWS and SRS

11P15 EPIGENETIC ANOMALIES AND FETAL GROWTH



Imprinting and disease

Embryonic Development

Ovarian teratoma
Hydatiform mole

Intra-uterine growth

Beckwith-Wiedemann Syndrome
Silver-Russell Syndrome
Fetal programming of adult
disease?
Pre-eclampsia?

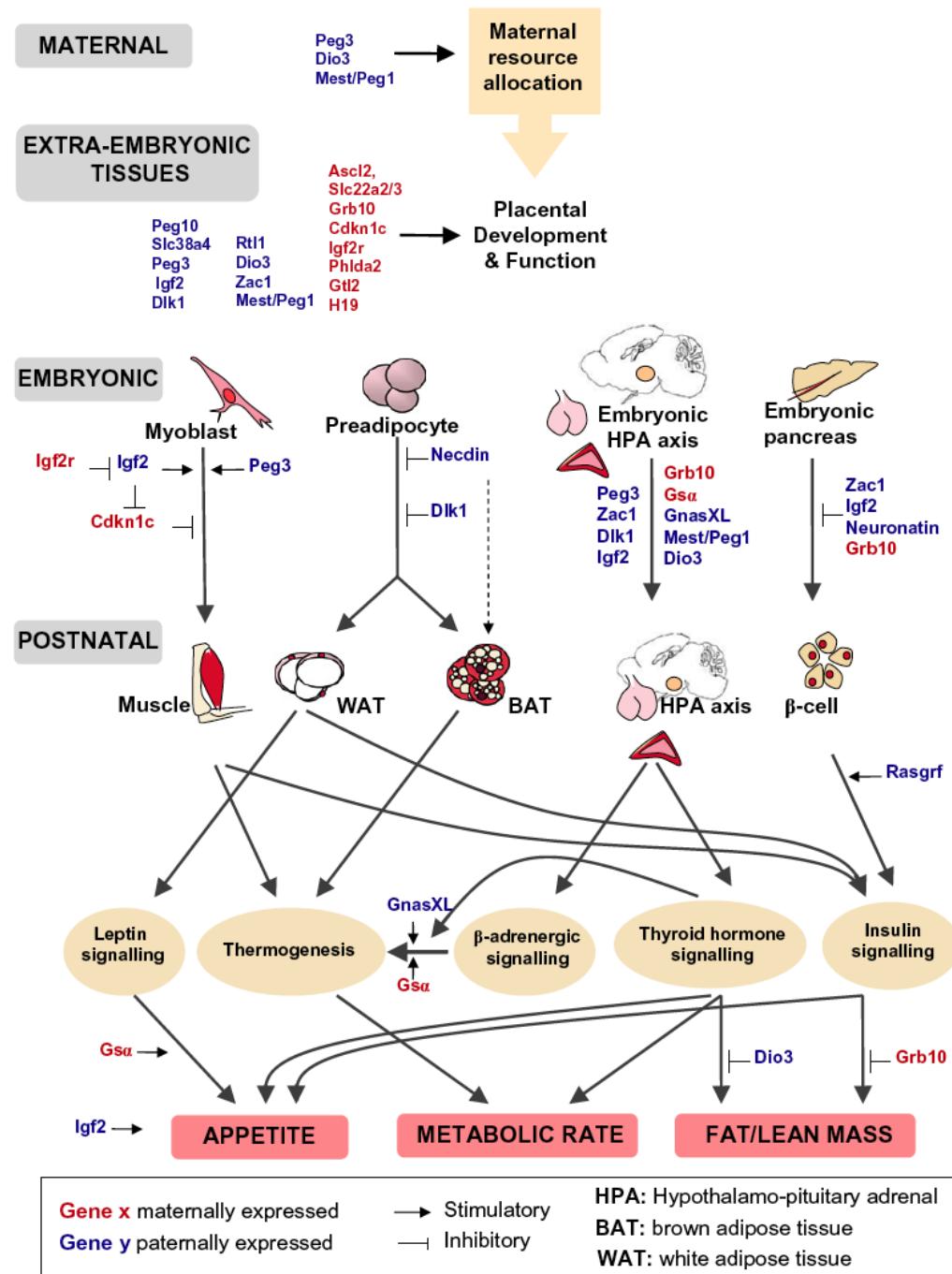
Behaviour and Brain:

Prader Willi-Syndrome
Angelman Syndrome
Turner's Syndrome
Tourette's syndrome?
Manic depression?
Schizophrenia?
Maternal behavior (in
mice)

Endocrinology and metabolism:

Albright hereditary osteodystrophy
Pseudohypoparathyroidism 1A
Transient neonatal diabetes
mellitus
Type I and Type 2 Diabetes?
Obesity?

A network of imprinted genes regulates key aspects of mammalian physiology



Summary of Part 2. Deciphering the molecular basis of imprinting

- *The quest for the germline imprint:*
 - DNA methylation:
methyltransferase knock-outs
- *“Reading” the imprint at Imprinting Control Regions (ICRs):*
 - CTCF (e.g. Igf2/H19)
 - long non-coding RNAs (Kcnq1ot1 or KvDMR1)
- *Disruption of ICRs has consequences for imprinting regulation and is associated with disease*

Why imprinting?

“Conflict of interests between parents regarding fetal growth rate”

Father

Fetal survival
Larger birthweight
restraint



Mother

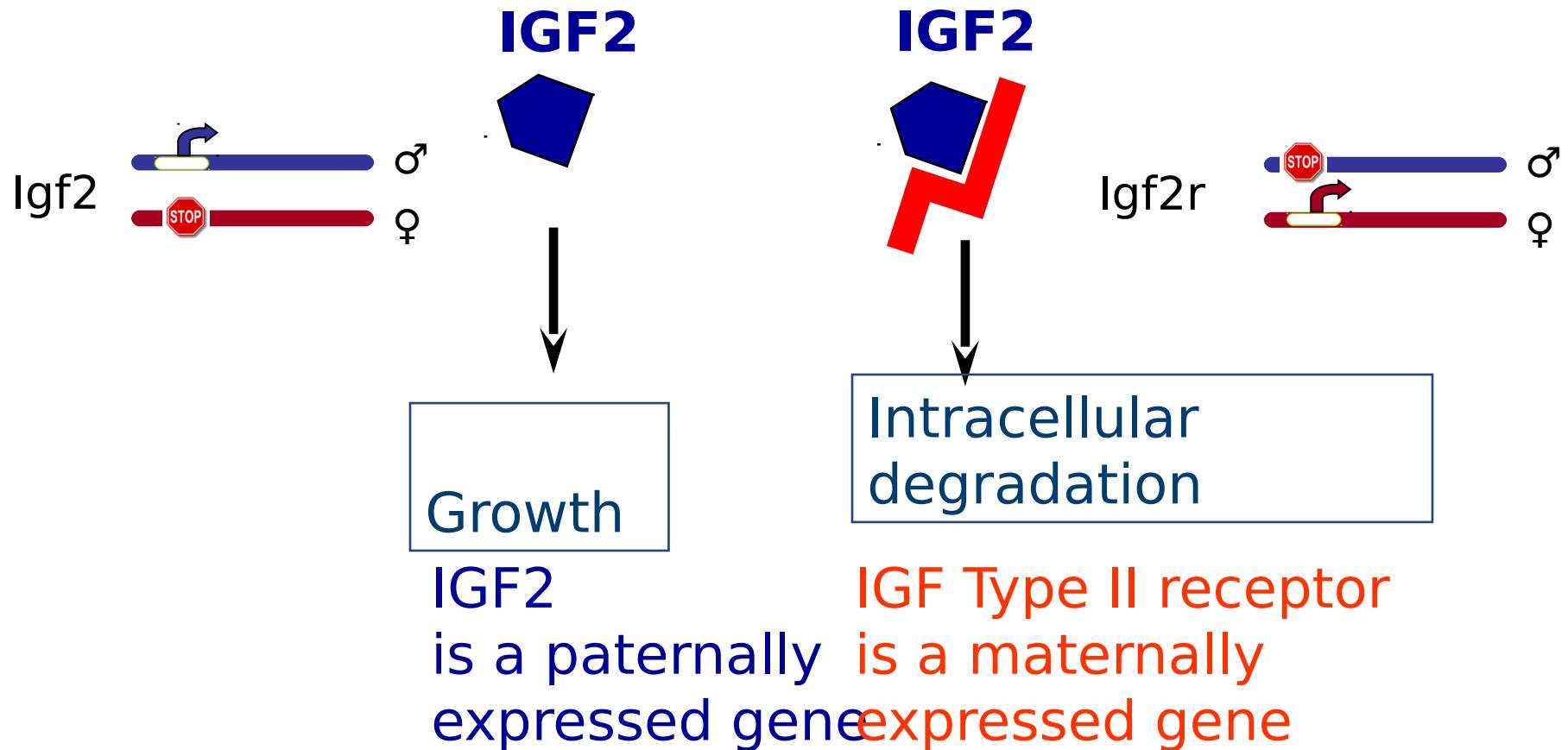
Mother survival
Fetal growth

e.g.

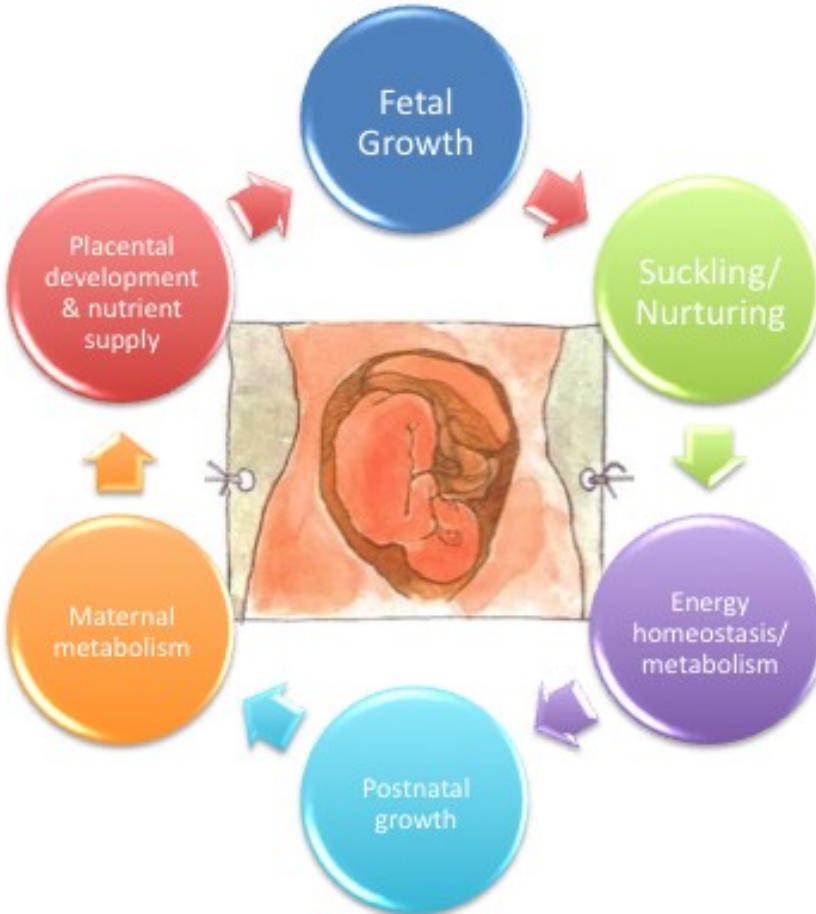
Mom's and
Pop's DNA
Punch-up

Haig & Graham (1991) *Cell* 7

An Arms Race



Imprinting and resource transfer from mother to offspring



Paternally expressed genes

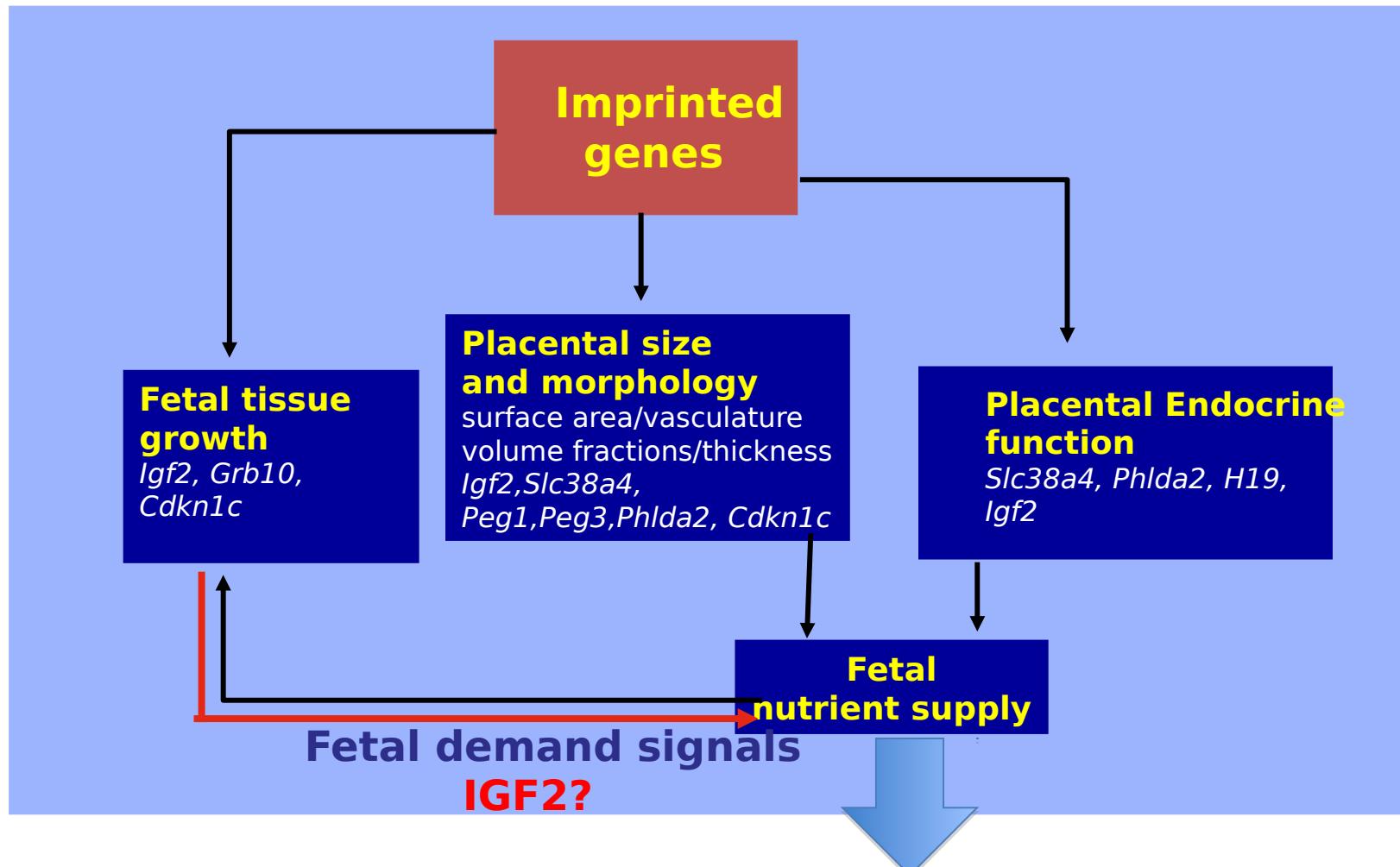
- promote resource transfer

Maternally expressed genes

- conserve resource transfer for future reproductive benefits

Antagonistic ‘interests’ of parental genomes are out in the fetus (demands)
In the placenta (supply)

Regulation of supply and demand by imprinted genes



Genomic Imprinting

- Confers a functional asymmetry between parental genomes
- Epigenetic modifications in the germline and embryo cause imprinted genes to be expressed either from the maternal or the paternal chromosome.
 - The mammalian oocyte is not truly totipotent due to parental genome asymmetry
 - Currently ~ 150 genes identified in mammalian genomes
- Major effects on fetal growth, placenta and postnatal behaviour
- Defects lead to disease and cancer
- Important features of epigenetic gene regulation first identified in imprinted genes